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ORIGINAL ARTICLES

APPARATUS USED IN THE ESTIMATION OF BASAL METABOLISM*

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THE increasing interest in basal metabolism as a means of diagnosis has raised many questions as to the type of apparatus most suitable for this test. The clinician naturally craves a small portable apparatus which can be carried from house to house and used with the same ease and accuracy as his blood-pressure apparatus and his clinical thermometer. Unfortunately this demand can at best be but partially satisfied, as all appliances so far devised involve the handling and measurement of gases; this includes the vexing problem of corrections for temperature, barometric pressure, and water vapor,—factors with which the clinical observer has never had to contend.

In designing apparatus for clinical respiratory work, portability seems to have become the prime requisite. While the idea is fascinating, in reality nothing detracting from the accuracy of the apparatus should be sacrificed on this score. The patient who is too ill to be moved to a laboratory, or to the physician's office, is not the type of case in which basal metabolism estimations are of value. Those using portable apparatus rarely transport them: almost invariably the test is done in the clinician's office or, in the case of a hospital, a suitable room is selected and the patient brought there for the test.

Basal metabolism is an expression of the heat production of the body while completely at rest and in the post-absorptive state, such a condition as is found upon awakening in the morning. The brilliant and painstaking work of Voit, Pettenkofer, Atwater, Benedict, Lusk, DuBois, and their many coworkers has demonstrated that the heat production can be indirectly determined by measuring the oxygen consumption, the heat value for the latter being derived from the respiratory quotient.

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It is necessary, therefore, to determine the amount of oxygen consumed and the amount of carbon dioxide produced in a given time. To accomplish this, two methods are in vogue. In the "open-circuit" method, the subject inspires atmospheric air and expires into a gasometer through a series of tubes, mask, and valves. The expired air, having been measured and its volume corrected, is analyzed for carbon dioxide and oxygen; the amount of oxygen absorbed is calculated on a basis of the ratio of expired nitrogen to atmospheric nitrogen. Dividing the volume of carbon dioxide produced by the oxygen absorbed gives the respiratory quotient, which has a known heat value for each liter of oxygen. In this manner the total calories produced per hour may be calculated. Accepting the heat production as practically proportionate to the surface area determined by the DuBois and DuBois formula,[1] the calories produced may be expressed in terms of calories per square meter of body surface per hour. This result, the basal metabolism, is usually reported as the percentage above or below the average of the DuBois normal standard.

The "closed-circuit" method requires the subject to rebreathe from a circulating current of confined air, rich in oxygen. The carbon dioxide and water vapor are removed by suitable absorbers and the consumed oxygen is represented by the decrease in volume, or by the weight of oxygen required to maintain the original volume. The carbon dioxide produced can be determined by weighing the absorbers, and the remaining calculations are the same as in the open-circuit method. This principle has been used by Benedict[2] in developing his "clinical respiration apparatus" and later, the "portable respiration apparatus." It has also been applied in simplified apparatus as those of Jones,[3] and Sanborn;[4] in these appliances the carbon dioxide is removed by absorbers and the consumed oxygen determined directly by the decrease in volume of the rebreathed air, which is almost pure oxygen. The carbon dioxide is not measured. The respiratory quotient is assumed to be 0.82, giving a constant heat value for oxygen.

In equipping the respiration laboratory of the New York Post Graduate Medical School and Hospital, we were greatly influenced by the broad experience of Boothby and Sandiford[5] in the clinical application of the test. Their arguments in favor of the open-circuit or gasometer method for institutional work seem to be sound, and, despite the bugbear of gas-analysis, the method is eminently satisfactory. In this procedure the expired air is collected under the most favorable conditions. The subject reclines in a pleasantly furnished room adjoining the laboratory which contains the entire apparatus; the face mask is adjusted, and the test started after the gasometer has been completely washed out with expired air. The test is started and stopped during inspiration without the patient's knowledge, and without his undesirable cooperation. No skill is required in this part of the procedure and, once the apparatus has been tested, the danger from leaks is negligible.

After the expired air is collected and measured in the gasometer, multiple samples can be kept in suitable containers and the gas-analyses repeated, even after an interval of several days, if there is any question of the accuracy of the results.

By the use of Boothby and Sandiford's calculation sheets[5] each step in the procedure can be checked by a second assistant, thereby greatly decreasing the chances of technical error. Logarithms, whose name alone has probably inspired dread in the minds of the uninitiated, is an invaluable means of accurately carrying out routine calculations. The use of the tables for multiplication and division can be mastered at one sitting, and it is greatly to be regretted that this valuable aid is not in the hands of every one attempting to do scientific work. Employing the Haldane apparatus[6] or one of its modifications,[7] the determination of the carbon dioxide is rapidly carried out before the oxygen analysis. With but slight additional work we are given a much broader view of the metabolic processes, the actual respiratory quotient is determined, the true heat value of the oxygen known, the basal metabolism most accurately estimated and fields thrown open for intensive studies in metabolism. Benedict[2] has stated that "only that form of apparatus which permits exact determinations of the respiratory quotient as well as of the total metabolism, need be seriously considered for the best clinical work."

The accuracy of the gasometer method is unquestioned, it is chiefly criticized for its lack of portability and for its time-consuming gas analyses which place it far beyond the reach of the great majority of physicians. This is quite true, but it is equally true of complement-fixation tests, the colloidal gold test, chemical analysis of the blood, and many other laboratory procedures, all of which are time-consuming and require special skill not possessed by the average clinician. The chief use of basal metabolism tests at the present time is in the diagnosis and prognosis of thyroid disease; the result of the test frequently determines when serious operative procedures can be undertaken with least risk for the patient,—certainly time and labor are paltry considerations under the circumstances. Boothby and Sandiford[5] have shown that the technic of gas analysis can be acquired by any intelligent high-school girl, and that the time required for the test is well within one hour. The entire procedure can be carried out by a well-trained technician under proper supervision. The "closed-circuit" method, when the respiratory quotient is determined, is an accurate means of estimating the heat production, and in skilled hands has resulted in the accumulation of valuable scientific data. Its use in routine institutional work, however, calls for extreme care and attention, first in testing the apparatus for leaks and secondly in making the determination. The oxygen absorbed is represented by any change in volume of the enclosed air; leaks in or out of the apparatus, incomplete absorption of carbon dioxide, and retention of air in the lungs or stomach at the beginning or end of the test will directly affect the results. Any check on the accuracy of the determination can be made only by again subjecting the patient to the test, and any check on the observations must be made by a second observer at the time of the test. It would seem that the time and skill required to conduct a number of tests with this type of apparatus greatly exceed that of the open-circuit type, while the difficulty in checking the observations renders it less desirable in routine institutional work.

The simplified types of closed-circuit apparatus[2, 3, 4] in which the only

estimation is that of oxygen consumption, doubtless have a wide field of usefulness, but this field should be closely defined and radical attempts, either medically or surgically, to correct the patient's metabolism should be instituted solely on the most careful observations using the most accurate method available. The simplified appliances have been introduced to the clinician as useful aids in diagnosis, but it is questionable whether they should be used as a means of surgical prognosis, or of conducting original research in metabolism.

RESPIRATION LABORATORY OF THE NEW YORK POST GRADUATE MEDICAL SCHOOL AND HOSPITAL

In planning a new department for a large institution, one soon discovers that space is the deciding factor, and that one must design and assemble the

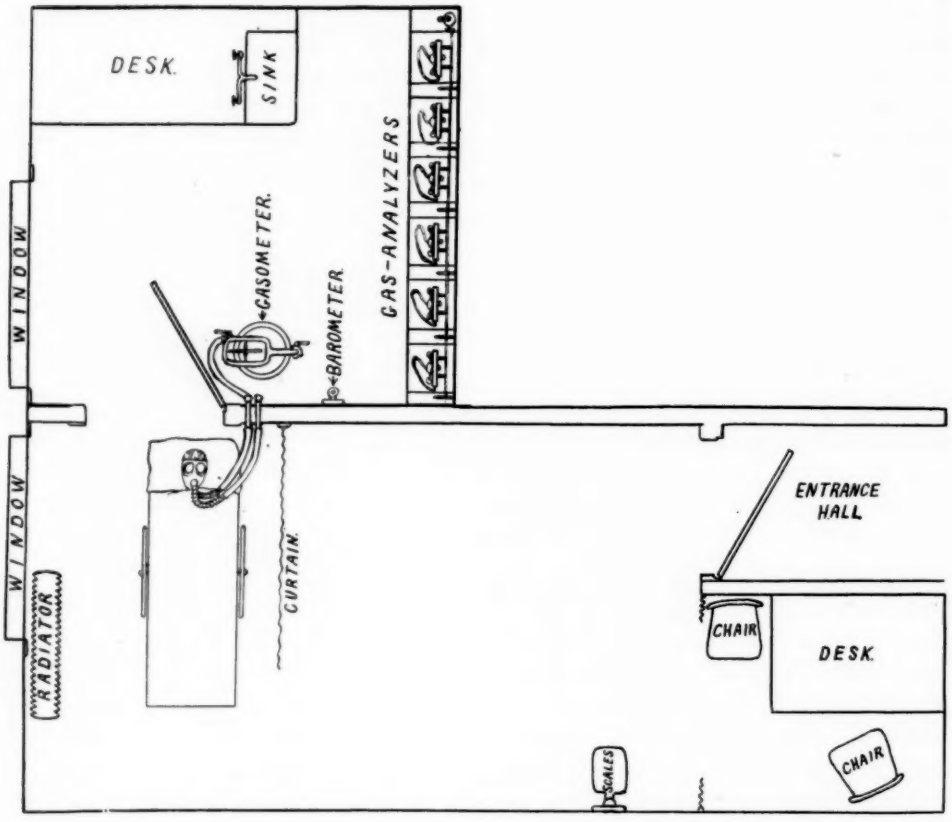

Fig. 1.

equipment with this limitation ever in mind. Our experience in this undertaking was no exception to the rule, but the very restriction imposed proved to be a blessing in the end, as it resulted in the assembling of a very compact and workable outfit for metabolism estimations. In general, the apparatus and mode of procedure do not differ from those frequently described;[6, 8] but in the particular design and assembling of the appliances, innovations have been

introduced which add to the accuracy and ease of manipulation, and seem to afford a sufficient excuse for a detailed description at this time.

Having decided upon the gasometer method as being the most suitable for routine institutional work, the first consideration was the handling of the patients; this must be done expeditiously, and in such a way as to allay their fears and to permit of complete bodily rest for a period of at least half an hour before the test is started. Two small rooms were placed at our disposal, the smaller, measuring 8 ft. by 8 ft. was set aside for a laboratory, the larger, 8½ ft. by 20 ft., was selected as an office and rest room for the patients. This room is finished in French gray with blue draperies and the hospital atmosphere is avoided as much as possible. Couches for the patients were dispensed with in favor of adjustable wheel-chairs, the latter, when comfortably padded prove very satisfactory, as the patient can remain in one chair from the time he leaves his room until his return at the end of the test. In

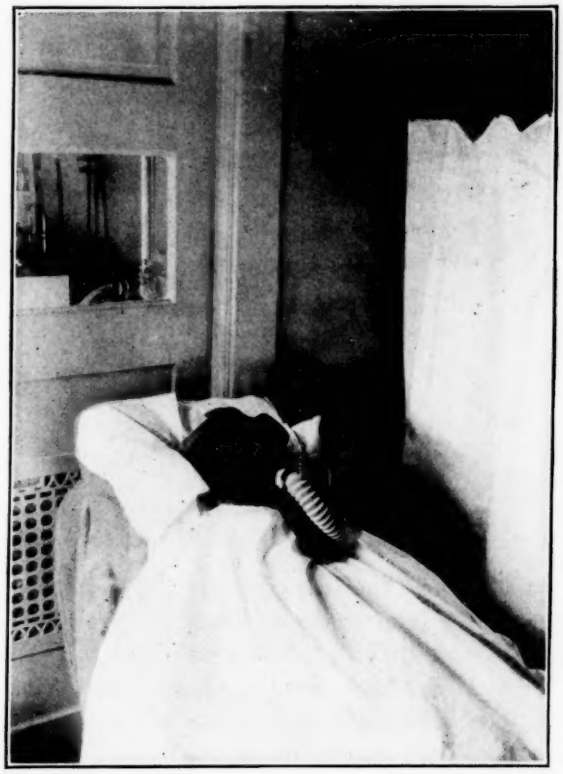

Fig. 2.

our rest room, three patients can be accommodated at one time, the chairs being moved into position for taking the test without disturbing the occupant. The general arrangement of the rooms is shown in the accompanying plan (Fig. 1).

It will be seen that with the exception of the mask and short lengths of air-tubes, the entire apparatus is placed in the laboratory. The door, which

contains a small observation window, affords the observer easy approach to the patient, so that one person can manipulate the gasometer and at the same time observe the patient.

MASK

The large number of breathing appliances, which have been introduced for use with respiration apparatus, is an evidence of the difficulty experienced in finding something suitable for this work. Some device is needed which can be rapidly fitted to any patient, which will permit of his normal type of respiration, which will accurately conduct the inspiratory and expiratory air without offering resistance, and which can be worn in comfort and without the danger of leaks. The gas mask[9] of the French army fulfills all of these requirements, when used in conjunction with the rubber flutter-valves introduced by the British during the late war. The mask (Fig. 2) is made of thick rubber, covers the whole face, and presents broad surfaces which closely

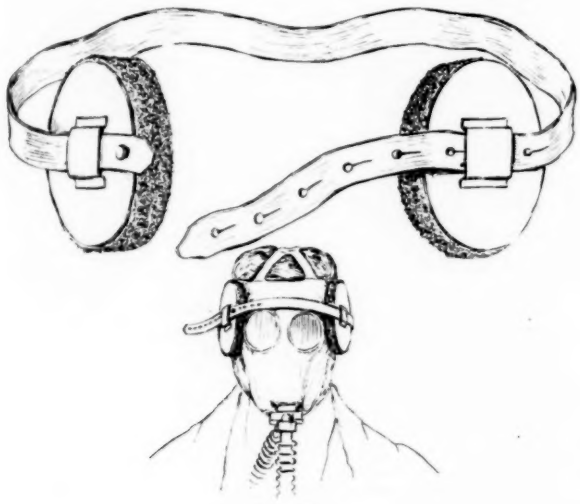

Fig. 3.

engage the forehead, sides of the face, and jaw. The tissues in these regions are well supported by the bony framework of the face and the mask readily adapts itself to these fixed surfaces. It is held in place by elastic straps passing around the head. With emaciated subjects, leaks may occur above or below the zygoma, in this area the pull of the straps is in the same plane as the surface of the face. In such instances, the leaks are readily overcome by placing 5-inch rubber sponges over these areas of the mask and binding them in place with a 3-inch bandage. In place of a bandage for this purpose, the sponge holders shown in Fig. 3 are more satisfactory; the sponges are cemented to thin metal plates which are adjustable on a broad strap passing around the head. In this mask the incoming air is directed upward towards the windows, the opening of the expiratory tube being opposite the nose and mouth; this insures complete ventilation of the space and no discomfort results. When applied to some individuals, the chin pad permits the mask to

press on the throat; this can be readily overcome by drawing the mask away from the chin by means of a cord fastened to the tube-connections on the mask, and tying the cord to a hook directly over the subject's head (*D*, Fig. 4). With this cord the mask can be comfortably adjusted and it has the added value of preventing movement on the part of the patient. Following the test, the mask and attached tubes are removed from the pipes (*C* and *E*, Fig. 4) passing through the wall, and are readily cleansed with soap and water.

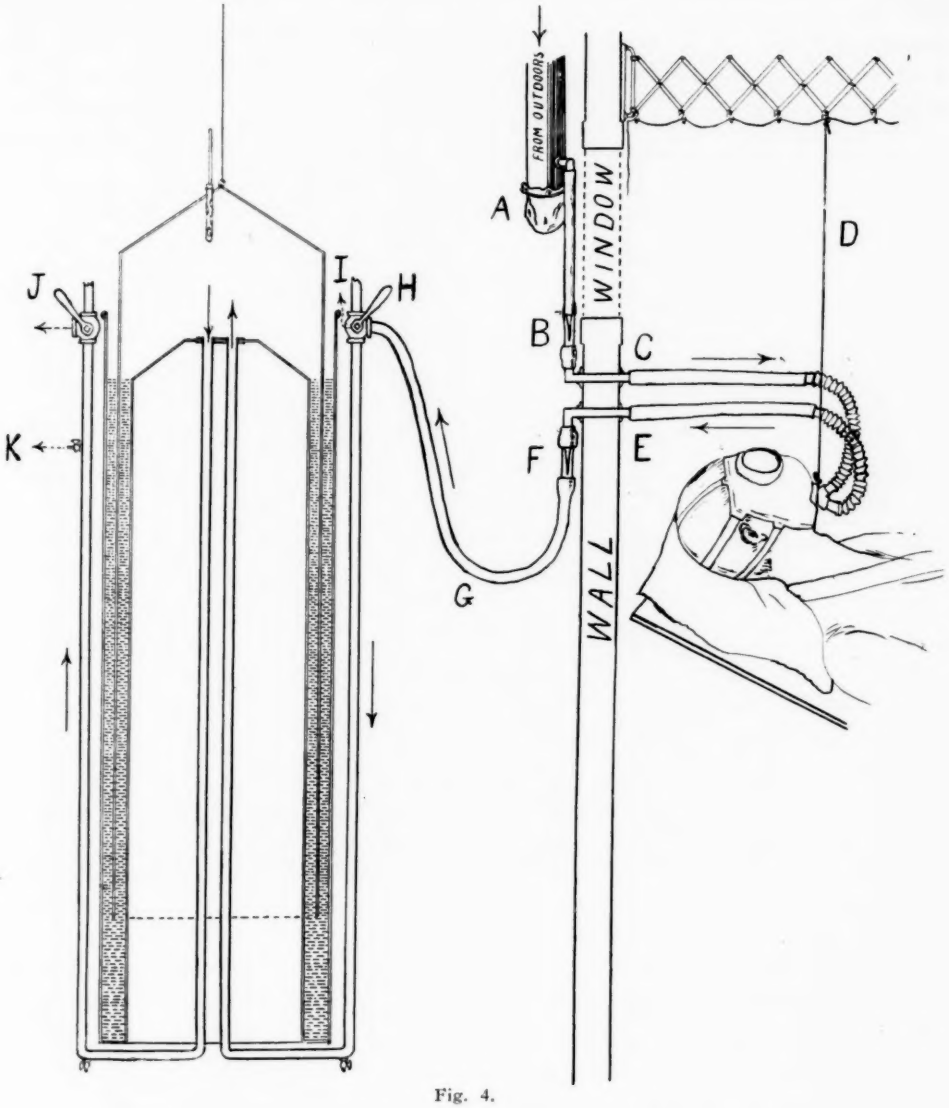

Fig. 4.

PIPES, VALVES AND CONNECTIONS

Outdoor air is brought to the laboratory through a 4-inch galvanized sheet iron drain pipe. This pipe terminates 2 feet above the valves, the open end being closed by a rubber bathing cap (*A*, Fig. 4) held in place by a rub-

ber band. The cap takes up the pressure of gusts of wind and prevents its blowing through the valves. A 2-foot length of 24 mm. rubber tubing leads from the large pipe to the inspiratory valve; this is for the purpose of trapping any expired air which might backlash during expiration. The arrangement of the valves is shown in Figs. 4 and 5; they are enclosed in flattened glass cases which are mounted directly on the laboratory ends of the wall tubes (*B* and *F*); the latter have elbows directed upward on the inspiratory and downward on the expiratory,—this permits both the flutter-valves to hang

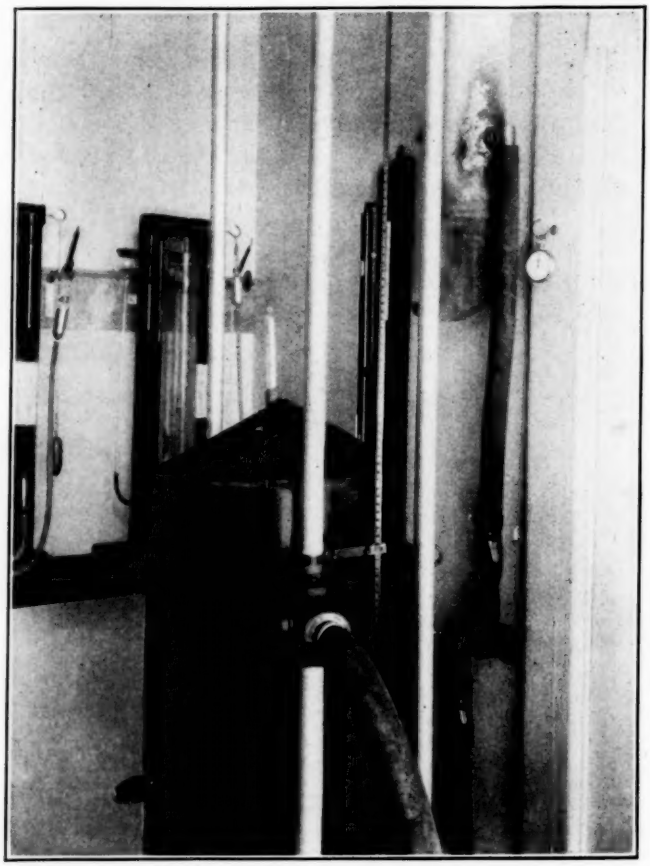

Fig. 5.

downward, in which position they functionate most favorably. From the lower end of the expiratory valve-case, a 24 mm. rubber tube (*G*) leads to the gasometer.

GASOMETER

The gasometer (Fig. 6) is designed as a stationary piece of laboratory apparatus; its ball castors, however, permitting it to be moved about the room should this be desired. For clinical purposes a gasometer of 90 to 100 liters capacity is the most serviceable. As the volume is measured by the rise of the gasometer bell, it is evident that for a given volume, the longer the

bell the more accurate the reading. The bell of this apparatus is 100 cm. in length and has a diameter of 34.59 cm.; measuring the rise of the bell to the nearest half-millimeter, a change in volume of 47 c.c. can be detected. The apparatus is 8 feet high and occupies no more floor space than an ordinary chair. The framework is rigidly constructed of iron pipes and castings, at the top it supports the counterbalance wheel, on its flat base rests the copper water-bath. The base consists of a malleable casting containing inlet and outlet passages for air; it is fitted with three adjustable legs for leveling the apparatus, and is threaded to receive the supporting and air pipes of the framework. Extending upward from the center of the base and connected with its air passages are two one-inch pipes which pass through holes in the upper end of the obturator of the water-bath, the joints being made firm and airtight by means of plates. The air passages are continued as part of the framework to a height of 4 feet where three-way valves are placed; from these the piping extends to the casting forming the upper part of the framework. The grooved counterbalance wheel is of brass and has a diameter of 23 cm.; it is mounted on a steel axle with spindle bearings, the latter being adjustable on the upper casting of the framework. The wheel carries a grooved spiral from which is suspended a compensating weight; as the bell rises from the water and becomes progressively heavier, the leverage of this weight is correspondingly increased and in this way the bell is exactly counterbalanced in all positions.

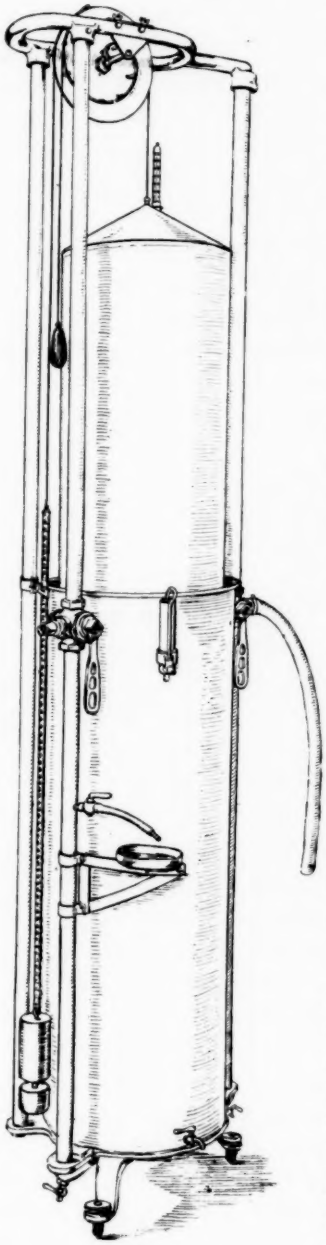

Fig. 6.

The bell is suspended by a piano wire which passes in the groove of the wheel and is continuous with a 100 cm. length of steel measuring tape, to the lower end of which the main counterweight, which is in two sections, is fastened by means of hooks. The tape, which reads in millimeters from below upward, passes through a suitable guide bearing a pointer at which the readings are made. The bell and water-bath are made of copper. The water-bath is circular in plan and has outer and inner walls, 4 cm. apart; the outer wall is 110 cm. high; the inner wall has a height of 100 cm. and with a truncated conical roof forms the obturator of the bell. The water-bath has a drainage cock near the bottom and a constant water-level attachment fastened to the

outer wall at a height of 100 cm. In use the bath is kept filled with water to this level. The outlet pipe has a sampling cock placed about a foot below the three-way valve; this pipe also supports a shelf for the sampling bottles. The bell carries a thermometer for recording the temperature of the air.

COLLECTION OF EXPIRED AIR IN GASOMETER

The patient is placed in a semi-reclining position in a wheel chair and is allowed to rest for twenty minutes or longer before the test is started. During this period the respirations and pulse rate are closely observed and the mask is not applied until the patient appears to be mentally and physically at rest. At this time one ascertains that the body temperature is normal. While the subject is resting one empties the gasometer by removing the lower portion of the counterweight and turning the valve *J* (Fig. 4) to communicate with the room air; the bell sinks until it rests on the obturator and the pointer will stand at zero on the measuring tape. The valve *H* (Fig. 4) is now turned so as to connect the air-tube (*G*) with the gasometer. The rubber cap (*A*) is removed for a few minutes, permitting the large intake pipe to fill with fresh air from outdoors; the cap is then replaced.

When the patient is sufficiently rested, the ends of the tubes attached to the mask are dipped in water and slipped over the brass pipes perforating the wall at *C* and *E*, care being taken that the inspiratory pipe leads to the upper openings in the mask. The mask is then applied by holding the chin portion in position and pulling the straps over the top of the head. When accurately fitted, the mask-cord (*D*) is tied to the overhead hook, holding the mask and head in the most comfortable position for the patient. From the time the mask is applied, the inspiratory air is drawn from outdoors through the pipes to the flutter-valve (*B*) and thence to the mask. The expiratory air passes from the mask, through the flutter-valve (*F*) and along the tubing to the small space between the top of the bell and the obturator; from there it passes through the outlet pipe and escapes into the room through the valve (*J*). In this way the entire dead space of the apparatus is flushed out with expired air.

As an extra precaution, the lower portion of the counterweight is replaced, at the same time closing the valve (*J*). The expirations are now caught in the gasometer. When the bell has been elevated 6 or 8 cm., the valve (*H*) is turned during inspiration, cutting off the gasometer and permitting the expirations to escape through the vent (*I*). The bell is now dropped nearly to the zero mark by removing the lower counterweight and opening the valve (*J*). This valve is then closed, the counterweight replaced, and the reading on the tape recorded to the nearest half-millimeter. One is now ready to start the test. The respiratory phases can be readily followed by observing the flutter-valves (*B* and *F*). During the inspiratory phase, the valve (*H*) is quickly turned to communicate with the gasometer and at the same time one presses the stopwatch and the collection is begun. The observation window permits the attendant to closely watch the subject during these procedures and record any movements. While the air is being collected, the respiration and pulse

rates are recorded at frequent intervals, and the patient is admonished to remain very still and encouraged by explanations that the test is nearing completion and that everything is very satisfactory. The duration of the test is determined by the subject's rate of ventilation. As a rule the gasometer is nearly filled at the end of 10 to 12 minutes, although this period may vary from 5 to 15 minutes. Calculation is facilitated by stopping on the minute, the quarter, or the half minute. This is accomplished by holding the stopwatch near the inspiratory valve (*B*) in such a way that both can be observed at the same time; the other hand grasps the valve lever (*H*); at the selected period of time, and during inspiration, the valve (*H*) is quickly turned cutting the patient off from the gasometer. Should the subject be expiring, one waits until expiration is completed before closing the valve. The watch is stopped at this time. One must avoid shifting this valve during expiration as it is very disconcerting to the patient. The reading on the steel-tape is now recorded to the nearest half-millimeter. The difference between this and the

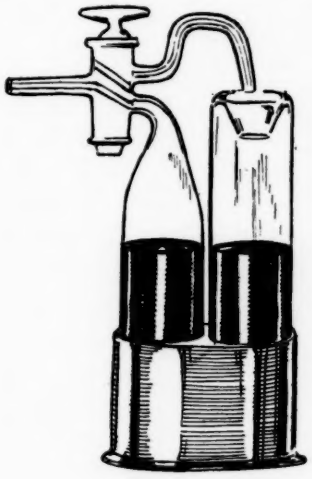

Fig. 7.

first reading tells the height in centimeters to which the bell has risen. As the bell is exactly counterbalanced in all positions, the enclosed air is under the prevailing atmospheric pressure. The lower portion of the counterweight is now removed leaving the air under positive pressure.

Since volumes of gas vary directly with the temperature and inversely with the barometric pressure, it is necessary to record these factors which will be used later in the calculations. The temperature is determined by a Centigrade thermometer inserted in the bell. A brass-scale mercurial barometer is used in recording the atmospheric pressure.

Samples of the expired air can now be collected in gas-sampling bottles.

GAS-SAMPLING BOTTLES[10]

The gas-sampling bottle (Fig. 7) consists of parallel glass cylinders communicating at the bottom through a small opening. The cylinders are firmly cemented into a metal case which forms a base. The top of one of the cylinders

has a funnel-shaped inversion; the other tapers off to a 2 mm. capillary tube to which a 3-way stop-cock is fused. The second opening in the stop-cock communicates with a capillary tube which bends over the inverted top of the first cylinder. The stop-cock terminates in a straight spout of capillary tubing 3 cm. long. The appliance is half filled with mercury, and before use is flushed out with 1 per cent sulphuric acid. A 6 cm. length of 3/16 inch rubber tubing is permanently attached to the spout.

In preparation for use, one sets the stopcock so that the spout communicates with the gas chamber (Fig. 7). The bottle is now tilted forward over a

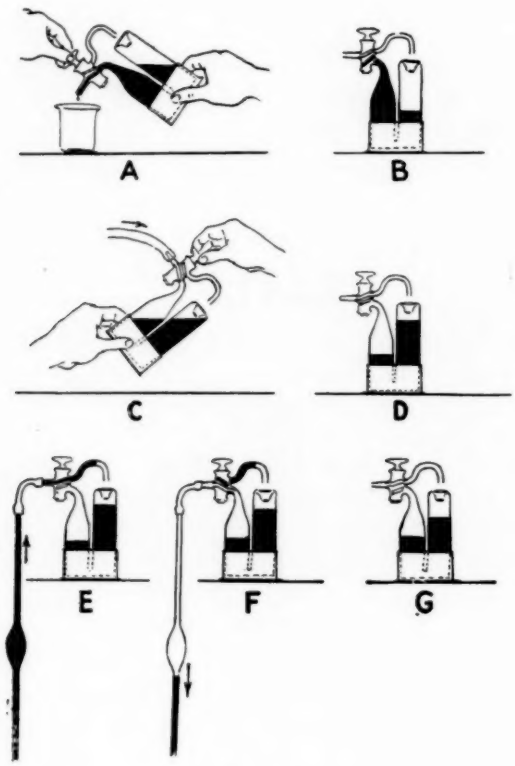

Fig. 8.

small beaker containing 1 per cent sulphuric acid, into which the rubber tube dips. The contained air is forced out by the mercury and when the bottle is tilted backward, acidulated water is drawn into the chamber. The apparatus is again tilted forward, this forces out the air and water. This position is maintained until mercury drops into the beaker (*A*, Fig. 8). The stopcock is immediately reversed; this leaves the gas chamber and the lower lead of the stopcock filled with mercury (*B*, Fig. 8) and the spout is in communication with the bent capillary tubing through which the air to be sampled is blown. Enough acidulated water remains in the chamber to saturate the air when the bottle is filled.

COLLECTION OF AIR SAMPLES FROM THE GASOMETER

Having removed the lower portion of the counterweight following the final reading of the gasometer, the enclosed air is under positive pressure. The prepared sampling bottle is now placed on the shelf and connected with the sampling-cock (*K*, Fig. 4). This cock is now opened and the air blows through the curved tube of the sampling bottle. Half the air in the gasometer is allowed to escape through the valve (*J*, Fig. 4); this is to insure a fair sample, as recommended by Boothby.[5] The stopcock of the sampling bottle is now reversed, the bottle removed from its shelf, and tilted backward (*C*, Fig. 8) and the compartment fills with the air from the gasometer. The stopcock is returned to its original position and the sample is trapped under pressure in the bottle (*D*, Fig. 8). Before trapping the sample, it is sometimes

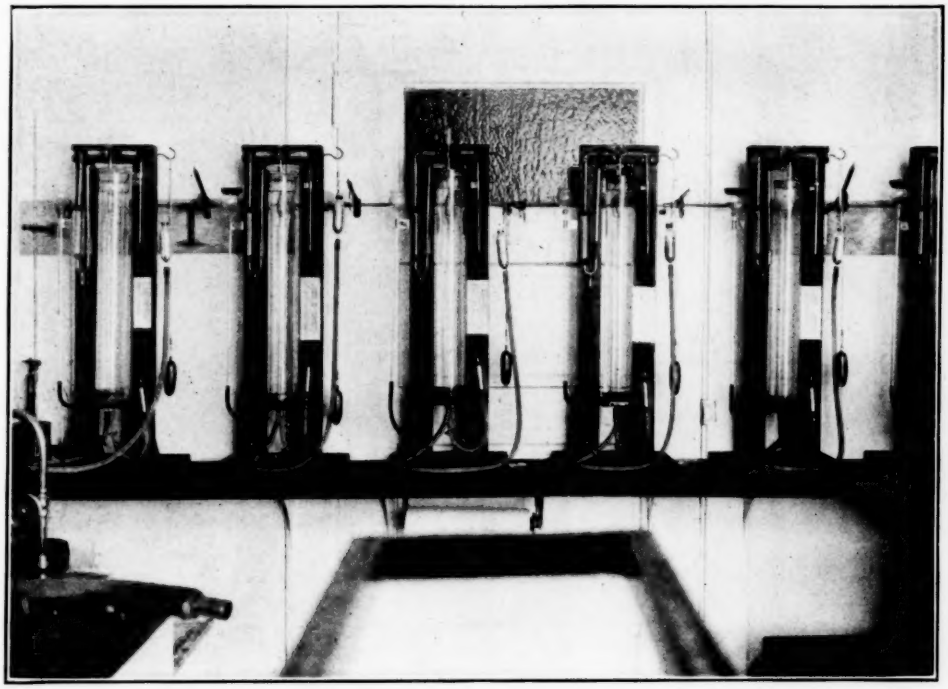

Fig. 9.

advisable to tilt the bottle backward and forward several times, at the same time allowing air to escape through the valve (*J*, Fig. 4); in this way one is certain of securing a good sample of the enclosed air. Two or three bottles can be filled in this manner, each bottle holding enough air for six analyses.

PRINCIPLES OF GAS ANALYSIS

The Haldane method is used in determining the amount of carbon dioxide and oxygen in the expired air. In principle, a volume of air is drawn into a graduated burette, where it is saturated with water vapor and measured; the air is then passed back and forth into a potash pipette, where the carbon dioxide is removed; it is returned to the burette and again measured, the difference

between this and the first reading representing the volume of carbon dioxide. The oxygen is removed in a similar manner by passing the air into a second pipette containing a potassium pyrogallate solution; after the oxygen is absorbed, the gas is returned to the burette and measured, this second loss in volume representing the oxygen in the sample of air.

In applying this method, several modifications of the original parts have been incorporated in the apparatus, the principal changes being the adoption

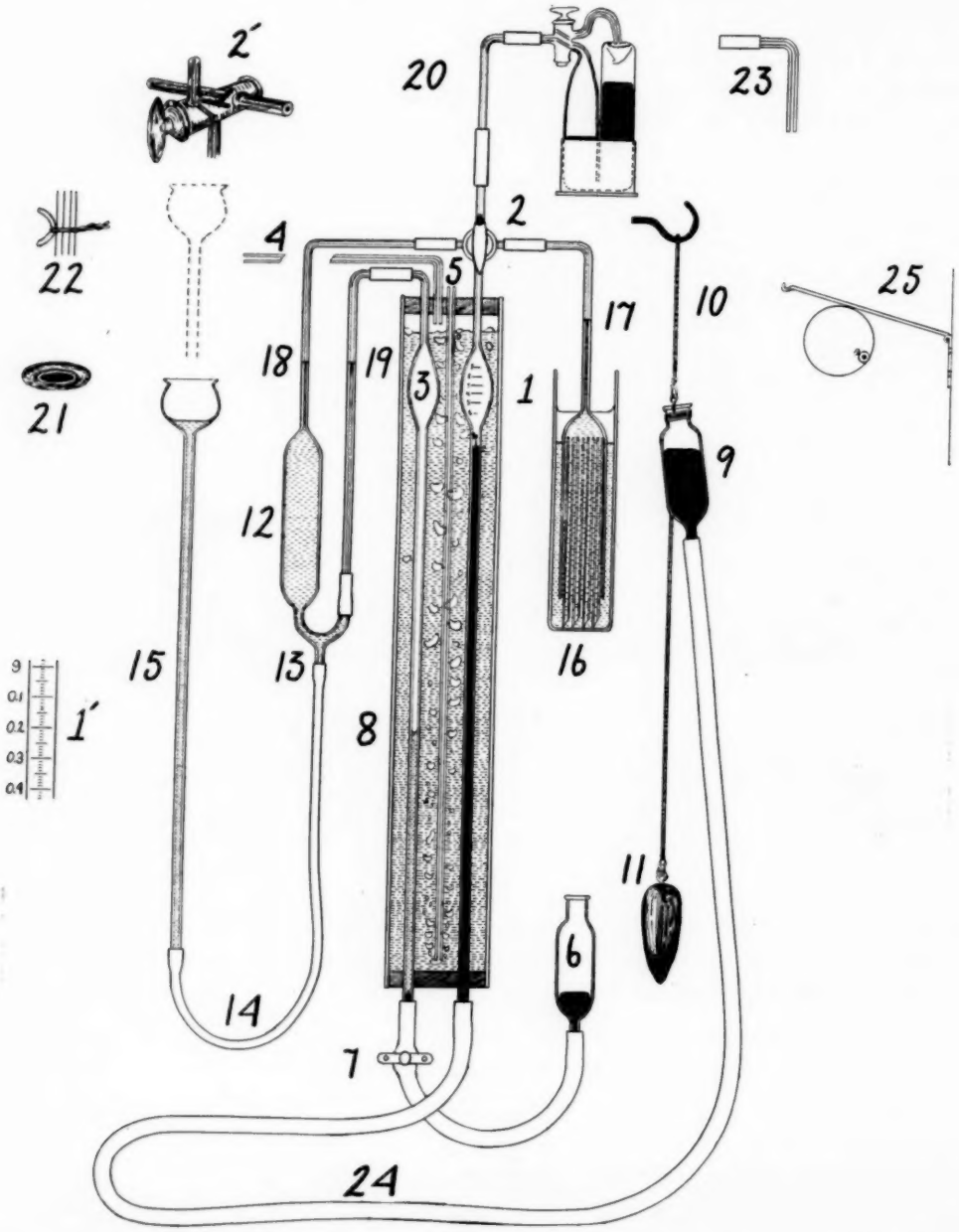

Fig. 10.

of Henderson's burette[7] with a four-way stopcock, and that author's type of oxygen absorber. These greatly simplify the assembling and cleansing of the parts and permit one to change the pyrogallate without disconnecting the apparatus.

DESCRIPTION OF APPARATUS FOR GAS ANALYSIS

The complete apparatus is pictured in Fig. 9, and shown diagrammatically in Fig. 10. It is mounted on a stout wooden support (Fig. 11), having grooves to receive the tubing and six brass clamps, by which the glass parts are held in place. On the top of the frame is a brass shelf upon which the gas-sampling bottle is placed. The space behind the water-bath is closed with ground glass, and behind this is placed a 12-inch "show-case" electric light bulb in a chain-operated socket, the chain being brought to the front of the support, so that the light can be readily switched on and off during the analysis. The table of

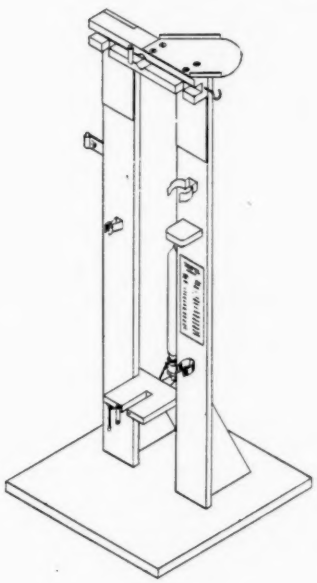

Fig. 11.

corrections for the burette is glued to the support to the right hand side of the ground glass. The frame carries a hook at the upper right hand corner, from which is suspended the mercury reservoir and its lead counterweight.

The assembled apparatus ready to be placed on its support, is shown in Fig. 10. It consists of a 10 c.c. burette graduated in hundredths (*1*) having a four-way stopcock at the top (*2*); this, along with a control tube of the same volume (*3*), is enclosed in a water-bath extending the full length of the burette. The water-bath is closed at top and bottom by rubber stoppers, through which the burette and control tubes protrude. The upper stopper has two additional perforations, one of which carries a suction tube (*4*) to the air space above the water, the second carries a tube (*5*) open at the top and extending to the bottom of the bath. When the first tube is connected with a suction pump, air is

kept constantly bubbling through the water, keeping it stirred up and equalizing the temperature of the enclosed burettes.

From the lower end of the control tube, a 24 cm. length of pressure tubing leads to a mercury reservoir (*6*). This tube carries a Hoffmann improved clamp (*7*) by which the lumen is occluded when the fluid in the conrol tube is set at the proper level (*8*). Attached to the lower end of the burette is a 95 cm. length of pressure tubing continuous with a mercury reservoir (*9*) which is suspended from the hook by a braided sash-cord (*10*) and counterbalanced by a conical lead weight (*11*). This weighs 447 gms. and exactly balances the reservoir and tube filled with mercury when the latter is halfway up the burette. When the reservoir is raised or lowered, there is enough friction between the cord and hook to hold the mercury at any level. For fine adjustment of the mercury level, the cord is grasped with the finger and thumb and rolled over the hook as one would turn a milled wheel.

The potash pipette (*12*) is placed to the left of the water-bath. Capillary tubes lead from the top and bottom of the pipette respectively to the left lead of the stopcock and to the upper end of the control tube. All the connections are made glass to glass and held tight by 4 cm. lengths of pressure tubing. The lowest opening in the pipette (*13*) is connected with a 21 cm. length of 3/16 inch sulphur-free black seamless rubber tubing (*14*). This is attached to the lower end of the thistle tube (*15*) which serves as a reservoir for the potassium hydroxide solution. This tube slides through a clamp on the frame, and by raising or lowering the reservoir equal changes of pressure are produced in the burette and in the control tube. The thistle tube is covered with a rubber cap (*21*) having a small perforation to permit the passage of air.

The right lead from the stopcock is continuous with a capillary tube which terminates in an inverted bell enclosed in a flat-bottomed tube (*16*). This bell is loosely filled with lengths of 3 mm. thin-walled glass tubing, beveled on the ends which rest on the bottom of the tube. When in use, this tube holds potassium pyrogallate covered with a 2 cm. layer of petroleum oil, which protects it from the air. This part of the apparatus constitutes the oxygen absorber.

The capillary tubes leading from the absorbers serve as manometers in adjusting the apparatus. A permanent mark (*17*) is cut in the tubing of the oxygen absorber 5 cm. above the bell. Sliding markers, as described by Henderson, are conveniently used on the manometer tubes leading from the CO_2 absorber (*18*, *19*). These are readily made by twisting a loop of fine brass wire around the tube, along which is laid a short length of a rubber band. This marker (*22*) is easily moved to the required position by grasping the ends of the rubber and the twisted wires; the rubber between the wire and the glass holds it firmly in position.

The upper lead from the stopcock is connected by pressure tubing to an elbow of capillary tubing (*20*), the horizontal part of which is 2½ cm. long and is at a height of 13 cm. above the shelf upon which the bottle rests.

In the construction of the apparatus, the capillary tubing used should be of uniform bore throughout and should be not less than 1.1 mm. and not

greater than 1.3 mm. in diameter. Care should be taken that constrictions do not occur where the tubing is joined to the bulbs, these retain fluid and interfere with the proper working of the apparatus. The burette, as supplied by the manufacturers, has a stopcock fused on the lower end. After recalibration this is cut off and the end of the burette smoothed with a fine file under water. The ends of the capillary tubing should be ground in this manner rather than fire-polished. The method of recalibrating the Haldane burette is completely described by Boothby.[5] The same instructions apply to the Henderson type, excepting the calculation of the mercury in the stopcock, which is not included in the volume of this burette. Before assembling the apparatus, the glass parts should be thoroughly cleansed with soap and water, then with warm cleaning solution, and finally rinsed in distilled water.

All the rubber tubing used in the construction of the apparatus should be scrubbed in soapy water in order to completely remove the talc. This can be done by coiling a pipe-stem cleaner, fastening it to a long wire and drawing it back and forth through the tubing; the rubber is then carefully rinsed and flushed out with one per cent sulphuric acid. In assembling the apparatus, a small amount of vaseline may be used in slipping the rubber over the glass tubing; this makes a very tight joint as it causes the rubber to adhere. In taking down an apparatus assembled in this manner, one must cut off these connections with a knife.

Black rubber grease is used on the stopcock. This is prepared by heating together equal parts of finely cut black rubber tubing, and lanolin. When the rubber has completely dissolved, the mixture is poured into an ointment jar and kept covered.

In preparing the absorbing solutions, it is convenient to keep a stock saturated solution of potassium hydroxide. For use in the potash pipette, this is diluted to a specific gravity of 1.15. The potassium pyrogallate solution is made by diluting the potassium hydroxide solution to a specific gravity of 1.55, and adding 10 gm. of pyrogallic acid to every 100 c.c. of the dilution. This absorber should be made up in quantity and is conveniently kept, well stoppered, in small citrate of magnesia bottles; the acid is first placed in the bottles, the potassium hydroxide solution added, and the bottles stoppered at once, making sure that the rubber washers are well in place. The solution improves with age. If one must use a freshly prepared solution, its efficiency as an absorber can be increased by exposing it to the air.

MANAGEMENT OF APPARATUS FOR GAS ANALYSIS

Preliminary.—Fill the water-bath with distilled water to within one inch of the upper stopper. Release clamp (*7*) at bottom of control tube. Place 2 c.c. of 1 per cent sulphuric acid in the control tube reservoir (*6*). Hold reservoir so that the dilute acid can barely be seen above the rubber tubing and superimpose about 6 c.c. of mercury,—this causes the acid to rise in the control tube. By raising or lowering the reservoir the acid can be brought to the level (*8*) in the control tube. This level is at the height of 8.5 c.c. on the burette. Close clamp (*7*) and replace the reservoir in its support.

Remove the barrel of the stopcock. Lower the mercury reservoir (*9*) until its opening is opposite to the bottom of the water-bath, and fill to the neck with mercury. Place the thistle tube (*15*) at a level slightly above that shown in the diagram and fill the pipette (*12*) with the dilute solution of potassium hydroxide until the level in the thistle tube is opposite to the mark (*18*). Cover the tube with a rubber cap (*21*). Fill the oxygen absorber (*16*) with the solution of potassium pyrogallate to a level with the top of the bell, and superimpose a 2 cm. layer of petroleum oil.

Grease the barrel of the stopcock with the black rubber mixture, care being taken that the holes are not occluded. Place the barrel in position and turn with gentle pressure until the burette is in communication with the intake tube (*20*). The black mark* on the stopcock handle indicates the lead which is in communication with the burette. Attach glass elbow (*23*) to the intake tube (*20*) so that the former points downward, and dips into dilute sulphuric acid contained in a beaker held beneath the tube. The burette is now flushed out with the acid by raising and lowering the mercury reservoir (*9*). The burette is then emptied by raising the reservoir until a few drops of mercury fall into the beaker. Remove the glass elbow (*23*).

The volume of air in the control tube is adjusted so that, when the meniscus is at the mark (*19*), the acidulated water in the control tube will be at a level (*8*) opposite to the 8.5 c.c. mark on the burette. This is accomplished by releasing the clamp (*7*) and raising the reservoir (*6*), causing air from the control tube to escape into the pipette (*12*). This procedure is repeated until the fluids are at the proper levels. (It may be necessary to add more mercury in order to force the air over.) Clamp (*7*) is then closed and the reservoir returned to its support. The air is now removed from the potash pipette by lowering the mercury reservoir (*9*) until the mercury in the burette is below the bulb, the stopcock is then given a quarter turn to the left, and the air removed by a further lowering of the reservoir. The air in the oxygen absorber is removed in a similar manner by first turning the stopcock to the right. The levels in each case are set at their marks (*18*) (*17*) on the manometer tubes.

The tube (*4*) is connected with a suction pump and a stream of air is kept bubbling through the water-bath. Before one can use the apparatus, it is necessary to absorb the carbon dioxide and oxygen from the air which fills the capillary tubes between the fluids and the stopcock. This is accomplished by drawing about 9 c.c. of air into the burette. Now make a quarter-turn of the stopcock *to the right*, at the same time slightly raising the mercury reservoir (*9*) so as to put the air under positive pressure and prevent the possible sucking of pyrogallate into the stopcock and burette. Absorption is brought about by alternately raising and lowering the reservoir (*9*). This pushes the gas back and forth between the burette and absorber, and brings it constantly in contact with new surfaces of fluid. In doing this, the mercury should not be carried above or below the bulb of the burette. This procedure

*If the handle is not already marked, this can readily be done by dipping the tip in hot sealing-wax. The tip on the same side as the nearest hole in the barrel is the one to be marked.

is repeated 18 to 20 times. The reservoir is then carefully lowered until the pyrogallate enters the manometer tube. Now one connects the burette with the potash pipette. This is accomplished by making a full half-turn of the stopcock *to the right* at the same time slightly raising the reservoir (*9*), as before, to prevent sucking the alkali into the stopcock and burette. The air is now washed back and forth several times and returned to the burette with the above precautions. The burette is again put in communication with the oxygen absorber by making a full half-turn of the stopcock *in the opposite direction to the hands of a clock,* taking care to slightly raise the reservoir as before. This procedure is repeated several times until the readings of the burette remain constant.

Preparatory to reading the volume, the pyrogallate is brought to its mark (*17*) on the manometer; the burette is then connected with the potash pipette by a half-turn of the stopcock *to the right,* with the usual slight raising of the mercury reservoir. The level in the potash manometer is now set by rolling the cord back or forth over the hook; at the same time the volume of the control tube is returned to normal by raising or lowering the thistle-tube (*15*). In this way changes of volume, due to temperature changes, are overcome by equally increasing or decreasing the pressure exerted on the two volumes of gas by the head of fluid in the thistle-tube. In order to be sure that free communication exists between the burette and the manometers, one momentarily pinches the rubber tubing (at the locations of the figures *14* and *24*)and determines that the levels in the manometers balance freely with the mercury. A drop of fluid or grease in the stopcock may interfere with the free transmission of pressure and lead to gross errors in the analysis. These obstructions, if present, can usually be dislodged by repeatedly pinching the tubing (*24*). The levels being properly set at their marks on the manometer tubes, one switches on the light which is placed at the back of the water-bath and reads the burette to the thousandth part of a cubic centimeter by means of a magnifying glass. To do this accurately, one places the eye so that the graduation mark, below the bottom of the mercury meniscus, and its reflection from the mercury, are directly in line; the location of the top of the meniscus between the hundredths divisions is then estimated in tenths. A small amount of acidulated water inside the burette is necessary to insure that the air is saturated with water vapor. If one flushes the burette with the dilute acid immediately before each test, sufficient moisture remains to bring about this saturation. When not in use, the nitrogen remaining after the last analysis is kept in the oxygen absorber; the stopcock is turned so that the burette communicates with the intake-tube (*20*); the potash reservoir (*15*) is lowered; and the clamp (*7*) is opened so that the fluid drops in the control manometer tube. The apparatus can be left safely in this manner, without the danger of alkali being drawn into the burettes as a result of temperature changes.

Analysis.—The first step is to start the pump which applies suction (*4*) and keeps the water well mixed in the bath. The potash reservoir (*15*) is raised until the surface of its contained fluid is on a level with the mark (*18*) on the manometer. The reservoir (*6*) of the control burette is now lowered,

causing the potash to rise in the control manometer to the mark (*19*); the clamp (*7*) is then closed tightly and the reservoir returned to its support.

The glass elbow (*23*) is now attached to the intake tube (*20*) and the burette flushed out with acidulated water, as previously described. The elbow is removed and the next step is to place the sampling-bottle upon its shelf and to connect the spout closely to the intake-tube, seeing that the stopcock is turned so as to cause these parts to communicate with the curved tube of the bottle. Next it is necessary to remove the nitrogen from the oxygen absorber and to set the levels in the manometer tubes; this is done by raising the mercury reservoir (*9*) until the small amount of acidulated water in the burette has entered the stopcock; the stopcock is given a quarter turn to the right and the reservoir lowered until the meniscus is at the mark (*17*); the stopcock is then given *a half-turn to the right*, at the same time slightly raising the mercury reservoir, thus throwing the burette into communication with the potash absorber. The levels in the manometers are set (*18*) (*19*) and the burette is connected with the intake-tube by making a quarter turn of the stopcock *to the right*. The burette and intake-tube are completely emptied of air by raising the mercury reservoir (*9*); this causes the mercury to rise in the burette, forcing the air and most of the acidulated water before it. When the mercury has passed into the curved tube as shown in ***E*** (Fig. 8), the stopcock of the bottle is given a half-turn, the reservoir lowered, and the expired air passes into the burette as shown in ***F*** (Fig. 8). (In this manipulation, the reservoir is grasped in the hand and raised slightly forward from the hook, leaving the cord in position). The sample is washed back into the bottle and again drawn into the burette by raising and lowering the mercury reservoir. This air is considerably compressed, and in order to take a sample which can be measured at atmospheric pressure, one sets the mercury meniscus at about 9.2 c.c., the stopcock of the bottle is then given a quarter of a turn, and the reservoir lowered until the burette reads in the neighborhood of 9.85 c.c. Next, the four-way stopcock is carefully given a quarter-turn *to the left*, and the levels in the manometer tubes (*18*) (*19*) rapidly reset by manipulating the thistle-tube (*15*) and the mercury reservoir (*9*). The volume of gas in the burette is now read to a thousandth part of a c.c. in the manner already described.

The carbon dioxide is absorbed by alternately raising and lowering the mercury reservoir, not permitting the mercury to rise above, or pass below, the bulb of the burette. After passing the gas, in this manner, eight or nine times one resets the levels (*18* and *19*) and reads the burette. The gas is again passed five or six times and the volume recorded. If the volumes do not agree within 0.002 c.c., the procedure is repeated until check readings are obtained.

To absorb the oxygen, one gently lowers the mercury reservoir (*9*) until the potash is within an inch of the bend above the mark (*18*), the stopcock (*2*) is then given a half-turn *in the opposite direction to the hands of a clock*, at the same time slightly raising the mercury reservoir to avoid the danger of sucking pyrogallate into the stopcock and burette. The gas is passed about

eighteen times into the oxygen absorber. It is then passed twice into the potash pipette, in order to wash out the oxygen which was left in the capillary tube, and again passed into the oxygen absorber an additional nine or ten times. This combined procedure is repeated twice, the pyrogallate meniscus reset (*17*), the burette thrown into communication with the potash pipette, the levels (*18* and *19*) readjusted, and the volume read on the burette. Absorption should be continued until check readings are obtained.

These three volumes are now corrected according to the recalibration of the burette. The difference between the second and first readings represents the volume of carbon dioxide in the sample; and this difference divided by the first reading tells the percentage of this gas in the expired air. In a similar manner, to determine the percentage of oxygen in the expired air, one subtracts the third from the second reading, and divides the difference by the original volume.

Bank of Gas-analyzers.—The arrangement of a bank of six gas-analyzers is shown in (Figs. 1 and 9). A stout shelf, 8 feet long and 12 inches wide,

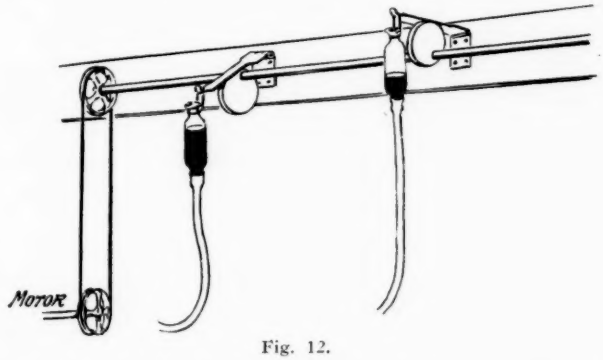

Fig. 12.

is firmly fastened to the wall at a height of 3 feet from the floor. This shelf supports the apparatus, which are placed with intervals of 4 inches between the bases. Beneath the shelf is a water-motor, belted to a speed-reducing gear (*1* to *68*), which in turn is belted to a countershaft (Fig. 12), supported from the wall at a height of 25 inches above the shelf. This countershaft carries a series of eccentrics which raise and lower the mechanical arms. A ¾ inch iron vacuum pipe, fitted with 6 pet-cocks, is placed along the wall, 2 inches above the shelf. From each of these pet-cocks, tubing leads to the suction-tube (*4*, Fig. 10) of the apparatus. Suction is applied to the vacuum pipe by means of a water suction-pump. Behind each apparatus is an electric plug to connect with the burette-reading lamp.

With this arrangement, one assistant can readily attend six analyzers. In using the mechanical arms to manipulate the gas, one grasps the mercury reservoir (*9*) in the right hand, the left hand detaches the cord from the reservoir and places the weight on the shelf. The reservoir is then hooked to the mechanical arm (*25*, Fig. 10). The arm has a movement of about 4 inches. A rate of 8 or 9 times a minute keeps the mercury moving from the bottom

RESPIRATION LABORATORY

Department of the Laboratories
New York Post-Graduate Medical School
And Hospital

Case No. 9569
Lab No. 27110
Name Rose Fraser, aged 22

Date 17-VI-21
Gasom. No. 1
Samp. Bottle No 4, 5, 6

Barometer 762.6 mm.
Temp. Gasom. 23. °C.

Gasom. End 89.9 cm. cm.
Start 0.3 cm. cm.
Diff. 89.6 (sub.) cm. cm.

Duration of test 10 m. 30 s. = 10.5 min.

CO2 Expired 3.31 %
CO2 Inspired 0.04 %
CO2 Produced (sub.) 3.27 %

O2 Inspired, corr. 21.15 %
O2 Expired 16.83 %
O2 Absorbed (sub.) 4.32 %

Log % CO2 produced 51455
Log % O2 absorbed 63548
Log. Resp. Quot. (sub.) 87907 - 0.76

Log. Fact. Gasom. 97313
Log. Gasom. diff. 95231
Log. Fact. S. T. P. D. 9523
Log. Total vent. (add) 87774
Log. Time 02119
Log. Vent. per min. (sub.) 85655 - 7.19 L.
Log. % O2 absorbed 63548
Log. O2 Absorbed (add) 49203 - 310.5 cc.
Log. Cal. value O2+log 60 4551
Log. Total Cal. per hr. (add) 94713 - 88.54 Cal.
Log. Surface area 15229
Log. Cal. per sq. m. hr. (sub.) 79484 - 62.35 Cal.

Cal. per sq. m. hr. (above nor.) 62.35.
Cal. per sq. m. hr. normal 37.
Cal. per sq. m. hr. (below nor.)
Difference (sub.) 25.35

Log. difference 4039
Log. normal 5682
Log. B. M. % (sub.) 8357

BASAL METABOLISM. +68 %

Analysis by JSG. Haldane No.: 4	Analysis by: JSG. Haldane No.: 5	Analysis by: Haldane No.:
9.780 -002 = 9.778	9.555 +0 = 9.555	
9.455 -001 = 9.454	9.235 +004 = 9.239	
.324	.316	
7.810 +001 = 7.811	7.625 +003 = 7.628	
1.643	1.611	
Log. CO2 diff. 51053 Log. O2 diff. 21564	Log. CO2 diff. 49969 Log. O2 diff. 20710	Log. CO2 diff. Log. O2 diff.
Log. sample 99025 Log. sample 99025	Log. sample 98023 Log. sample 98023	Log. sample Log. sample
Log. CO2 % 52030 Log. O2 % 22539	Log. CO2 % 51946 Log. O2 % 22687	Log. CO2 % Log. O2 %
CO2 % 3.31 O2 % 16.8	CO2 % 3.30 O2 % 16.86	CO2 % O2 %

Notes: Ht - 153 cm.
Wt - 104 lbs.
Surface Area - 1.42 Sq. M.
Resp - 22
Pulse - 128, 120

Avg. CO2 3.31 %
Avg. O2 16.83 %
CO2+O2 20.14 %

Readings by:
Checked by:
Calculations by:
First check by:
Second check by:

Fig. 13.

to the top of the burette bulb. The throw of the mercury is regulated by the speed, and by the amount of mercury in the reservoir.

CALCULATION OF THE BASAL METABOLISM

In calculating the basal metabolism, the scheme worked out by Boothby and Sandiford* has been adopted. In their calculations these authors use four place logs without the characteristics, and by arranging the various steps

*For the details of the calculations and the necessary tables, reference should be made to the book of Boothby and Sandiford.[5] The calibration of the gasometer is also detailed in this publication.

in their order of sequence, the entire calculation is made on one form-sheet (Fig. 13). This form has bracketed instructions for each step so that the danger of mathematical errors is largely eliminated. For expressing the volume of expired air at normal temperature and pressure, they have devised log tables of the factors which, at one step, make corrections for the brass-scale barometer, water vapor, temperature, and pressure. The triplicate analyses of the expired air are recorded on the same form sheet. Tables are given for computing the volume of oxygen inspired, based on a comparison of atmospheric and expired nitrogen. The oxygen absorbed is determined by subtracting the expired oxygen from the estimated inspired oxygen. One subtracts the carbon dioxide in atmospheric air (0.04 per cent) from that found in the expired sample, in this way determining the carbon dioxide produced. The ratio of carbon dioxide produced to oxygen absorbed is the respiratory quotient, and this value being known, by consulting the tables one can determine the number of calories produced as a result of the consumption of the determined amount of oxygen per hour. This heat production is expressed in terms of calories per square meter of body surface per hour,—the surface area being derived from DuBois height-weight chart. The basal metabolism is recorded as the per cent above or below the average normal standard of Aub and DuBois.[11]

SUMMARY

A detailed description has been given of apparatus* used in determining the respiratory exchange in man. The arrangement described is suitable for routine laboratory or institutional use in determining the basal metabolism. The particular features are the use of the full-sized gas mask, the special arrangement of rubber flutter-valves, a newly designed gasometer, the use of a new type of gas-sampling bottle in conjunction with the Henderson-Haldane gas-analysis apparatus, a detailed description of the construction and use of the latter, with several added mechanical features which greatly lessen the labor of gas analyses.

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*This equipment, complete or in part, may be obtained from the C. M. Sorensen Co., 177 East 87th Street, New York.

DISTRIBUTION OF URIC ACID IN THE BLOOD*

By Ruth C. Theis and Stanley R. Benedict, New York City

FOR the past two years as opportunity offered, we have been studying the distribution of uric acid between plasma and corpuscles, as well as the permeability of corpuscles to added uric acid. In the meantime Bornstein and Griesbach[1] published a paper dealing with the uric acid content of whole blood and plasma before and after incubation and after heating in a water-bath to 56° for one-half hour. By determining uric acid in the whole blood and serum and determining the corpuscle volume of the blood, they calculated the corpuscle uric acid on a basis of milligrams of uric acid per 100 c.c. of corpuscles. In their study of 20 cases they found 2 in which serum and corpuscle uric acid were equal on a volume basis; 13 in which serum uric acid was less than corpuscle uric acid; and 5 in which serum uric acid was greater than the uric acid in an equal volume of corpuscles.

We employed the Benedict[2] modification of the Folin-Denis method for uric acid determinations, using 10 c.c. of blood, and aluminium cream for the second protein precipitation instead of colloidal iron. Corpuscle volume was determined by the hematocrit. For the bloods from the wards of the Memorial Hospital we are indebted to Dr. William Stone; for bloods from the Roosevelt Hospital to Dr. William Lyle. The former bloods were defibrinated and the latter oxalated.

In a series of 104 cases we have determined sometimes both plasma and corpuscle uric acid, sometimes calculated corpuscle uric acid from the known corpuscle volume and sometimes calculated from the average corpuscle volume of 35 per cent. Finally the permeability of the corpuscles to added uric acid

Table I

Distribution of Uric Acid Between Plasma and Corpuscles

CASE NO.	WHOLE BLOOD URIC ACID MG. PER 100	SERUM URIC ACID MG. PER 100	CORPUSCLE URIC ACID MG. PER 100
1	3.4	3.1	3.4
2	2.8	3.0	1.8
3	3.6	3.4	3.7
4	2.6	3.0	1.8
5	6.0	6.4	2.3
6	2.7	2.6	2.8
7	4.8	4.3	4.7
8	2.9	2.6	2.6
9	3.7	2.6	3.8
10	3.1	3.5	2.6
11	3.7	3.4	3.3
12	3.9	3.3	3.1

*From the Huntington Fund for Cancer Research, Memorial Hospital, and the Harriman Research Laboratory, Roosevelt Hospital, New York.

was tested. Both defibrinated and oxalated bloods were used. From the figures which follow we can see that the various methods of approach serve to show that the relationship is a fairly constant one. Cases are divided almost equally between those in which uric acid is equally divided or those in which it is greater in the plasma.

The 12 cases shown in Table I represent those in which uric acid was determined on the corpuscles as well as whole blood and serum. Corpuscles were washed twice with .8 per cent NaCl. Fifty per cent of those bloods show an equal distribution with one exception; the others show that uric acid is higher (.9-4.1 mg. per 100) in the serum than in an equal volume of corpuscles.

TABLE II

DISTRIBUTION OF URIC ACID BETWEEN PLASMA AND CORPUSCLES*

Based on determinations in plasma and whole blood, and calculation from corpuscle volume.

CASE NO.	DIAGNOSIS	WHOLE BLOOD URIC ACID MG. PER 100	PLASMA URIC ACID MG. PER 100	CORPUSCLE URIC ACID* MG. PER 100	CORPUSCLE VOLUME %
12	Neurasthenia	3.2	4.9	–	34
13	"	1.7	3.2	–	30
14	"	3.4	4.2	2.6	46
15	Normal (?)	4.2	5.6	1.0	31
16	"	1.8	2.6	–	26
17	Pregnancy	3.4	4.3	1.7	36
18	"	4.4	5.5	3.2	46
19	"	2.2	3.5	–	41
20	"	3.5	5.5	–	33
21	Previous eclampsia	3.3	4.0	1.9	35
22	Ca. Uterus	3.6	4.8	–	17
23	Brain Tumor	4.7	6.7	–	31
24	Ch. Nephritis	5.8	6.9	1.3	21
25	Cardio. Neph:	2.8	2.1	4.6	28
26	Pregnancy	2.1	2.2		26
27	"	3.6	3.3		34
28	"	2.3	2.4		37
29	Normal	2.3	1.9		38
30	Syphilis	5.5	5.8		26
31	Arterio Sclerosis	1.9	1.9		28
32	Nephritis Decomp.	2.8	3.0		27

*Where the variation between the uric acid content of whole blood and of plasma does not exceed .5 mg. per 100 c.c. it is assumed that the uric acid content of plasma and corpuscles is equal for an equal volume.

Table II shows a series of 22 cases of oxalated bloods with corpuscle uric acid determined from the known corpuscle volume. Since a small error in reading of either whole blood or plasma uric acid would cause a very great difference in the calculated corpuscle uric acid, plasma uric acid that was 0.5 mg. per 100 higher or lower than the whole blood was considered equal. The corpuscle would then be the same also. These results may be summarized as follows: 8, or 36 per cent, give equal values; in 13, or 59 per cent, plasma uric acid is greater than corpuscle uric acid, with 7 negative in the corpuscles and 1 in which plasma uric acid is less than corpuscle uric acid.

Thirty oxalated bloods, where corpuscle volume was arbitrarily set at 35 per cent (average of corpuscle volume in Table I) show that 15, or 50 per cent, have equal values. Ten, or 33 per cent, have plasma uric acid greater than corpuscle uric acid, and 5, or 16 per cent, have plasma uric acid less than corpuscle uric acid. This is the only series in which there is such a large percentage of cases in which the corpuscle uric acid exceeds the plasma uric acid. Twenty cases of defibrinated bloods show 10, or 50 per cent, equal values; 9, or 45 per cent, with plasma uric acid greater than corpuscle uric acid, and 1 with plasma uric acid less than corpuscle uric acid.

Since each series showed practically the same relationship between plasma and corpuscles, we were interested to see whether the corpuscles are permeable to added uric acid. Uric acid was dissolved in lithium carbonate solution and was added to the blood in quantities of from 2 to 5 mg. per 100 c.c. of blood and left overnight. Whole blood uric acid, plasma uric acid before and after addition of uric acid and corpuscle volume were determined. Table III shows

TABLE III

PERMEABILITY OF CORPUSCLES TO ADDED URIC ACID*

CASE NO.	WHOLE BLOOD URIC ACID MG. PER 100	PLASMA URIC ACID MG. PER 100	CORPUSCLES URIC ACID MG. PER 100	CORPUSCLE VOLUME %	DISTRIBUTION OF ADDED URIC ACID
34	3.3	4.7	1.1	33	Plasma
35	2.5	3.7	–	32	"
36	1.8	2.5	–	26	"
37	1.7	2.4	–	27	"
38	2.4	4.0	–	30	Equally distributed
39	1.7	3.7	–	35	"
40	2.8	3.4	1.3	30	Plasma
41	2.7	4.6	–	40	Equally distributed
42	3.5	3.0		30	Plasma
43	4.2	3.7		30	"
44	2.4	2.6		28	Equally distributed
45	1.3	1.2		23	Plasma
46	2.2	2.5		34	"
47	1.5	1.8		26	"
48	2.6	3.1		29	"
49	3.9	4.4		34	Equally distributed
50	1.2	1.3		39	Plasma
51	.8	.8		32	"
52	5.3	5.5		36	"
53	1.7	2.0		33	Equally distributed

*Where the variation between the uric acid content of whole blood and of plasma does not exceed .5 mg. per 100 c.c. it is assumed that the uric acid content of plasma and corpuscles is equal for an equal volume.

the results of these determinations, the last column showing whether added uric acid was concentrated in the plasma or equally distributed. Sixty per cent of the 20 cases show equal values in the original blood and in 40 per cent plasma uric acid is greater than corpuscle uric acid, and 6 of these show no uric acid in the corpuscles. In 14 cases the added uric acid is all present in the plasma; 5 of these had shown the plasma of the original blood higher than the corpuscles, while in 9 the uric acid had been equally distributed. In 6 cases the added uric acid was equally distributed; in 3 of these the original uric acid had been equally distributed.

If we consider whole blood and plasma uric acids equal if there is a difference of .5 mg. between them in Bornstein and Griesbach's table as we did in our calculation, we find that 35 per cent of their cases have equal values; in 45 per cent serum uric acid is greater than corpuscle uric acid; and in only 20 per cent is serum uric acid greater than corpuscle uric acid. In our series of 104 bloods only 8 have a higher corpuscle uric acid, while all other bloods show either equal distribution or higher concentration in the plasma.

This holds whether the bloods are defibrinated and obtained from patients suffering from cancer and allied diseases (Memorial Hospital) or oxalated and obtained from a general hospital service (Roosevelt Hospital). As a matter of fact, out of the 42 cases in which corpuscle volume was determined by the hematocrit there were 13 in which there was no uric acid in the corpuscles. When uric acid is added to the blood, the plasma contains all the added uric acid in a large percentage of cases.

SUMMARY AND DISCUSSION

1. Uric acid was determined in plasma and corpuscles in 104 cases, 51 of which showed equal distribution; 45 showed plasma uric acid greater than corpuscle uric acid and 8 showed a greater amount of uric acid in the corpuscles than the plasma.

2. This relationship holds whether the blood is oxalated or defibrinated and does not depend on the pathologic condition.

3. Added uric acid did not penetrate the corpuscles in 70 per cent of 20 bloods studied. In 30 per cent of the cases the added uric acid was equally distributed between corpuscles and plasma.

The marked difference in permeability of the corpuscles of certain bloods for added uric acid is of interest, and suggests that other cells in the body may show similar differences in permeability. Such findings may tend to throw light on the questions involved in specific uric acid retention in the organism.

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[1]Bornstein and Griesbach: Biochem. Ztschr., 1919, ci, 184.
[2]Benedict: Jour. Biol. Chem., 1915, xx, No. 4.

VENTILATION, WEATHER, AND THE COMMON COLD*

A Study of the Prevalence of Respiratory Affections Among School Children and Their Association with School Ventilation and the Seasonal Changes in Weather

(Continued from page 610)

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ANALYSIS OF SICKNESS RATES IN INDIVIDUAL ROOMS AND SCHOOLS

As we have pointed out in the introductory remarks, great care must be taken in drawing conclusions as to the correlation of different facts from the average results of a group. In the study before us it will be necessary to inquire into the records of each school and of the individual rooms to see whether they agree uniformly with the characteristics of the group.

In Tables XI and XII are assembled records for each room covering the nature of air conditions and the amount of respiratory sickness.

It is noticeable that there is a wide variation in respiratory illness. Room 415 (Type B) at P. S. 22 in the second study had no absences whatever from respiratory illness. Room 311 (Type C), P. S. 115, in the second study, has a rate of 50.4. These represent the minimum and maximum limits. The range of respiratory sickness-in-attendance rates is even greater—from zero to 316.

Room 311, P. S. 59 (Type A) is the most congested in the first study, there being but 6.5 square feet of floor space per pupil. In spite of this crowded condition, the absence rate from respiratory disease is only 0.6—one of the lowest. On the other hand, Room 202, P. S. 165, being the least congested, with 19.6 square feet per pupil, has a respiratory absence rate of 37.0, a very high figure. Overcrowding does not inevitably lead to respiratory illness.

The average temperature of Type A rooms was about 59 degrees in both studies. This is much colder than the ordinary school room. In fact, it seems from our general experience entirely too cold for public school children, and yet, on looking over the absence rates, there is no indication that these children had more colds as a result. In fact, the average absence rate for the entire group is lower than the other two ventilation types, as has already been pointed out. In the first study there are only two rooms in Type A with rates over 20. There are three each in Types B and C. Respiratory sickness among those present in school is greater in Type A than in B, but less in A than in C.

In spite of the well intentioned efforts to balance the three types of rooms in the matter of schools and type of pupil, this could not be carried out to

TABLE XI

RECORDS OF INDIVIDUAL ROOMS IN FIRST STUDY

SCHOOL	ROOM	RESPIRATORY SICKNESS RATES			AV. TEMP.	AV. REL. HUMIDITY	PER CENT SESSIONS			PER CENT GIRLS	GRADE	SQ. FT. OF FLOOR SPACE PER PUPIL
		AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL			ESP. FRESH	ODOROUS				
Type A—Cold Open Window Rooms												
12	303	15	9.6	24.6	61.7	45	87	1		49	3A	15.5
	409	8.6	3.1	11.7	59.5	45	95	0		100	4B	13.5
	410	5.0	16.0	21.0	60.3	43	81	0		100	4B	13.8
39	202	11	0	11.0	56.9	52	96	1		0	3A	18.9
	203	14	.6	14.6	57.4	46	96	1		0	4A	13.6
	204	14	0	14.0	57.5	50	92	1		0	3B	15.1
	205	21	2.4	23.4	56.8	47	93	0		0	3B	15.0
	206	17	1.8	18.8	57.5	48	94	3		0	5A	14.4
	207	11	1.7	12.7	56.9	45	96	1		0	4B	11.7
59	311	.6	21.0	21.6	59.0	38	28	9		27	3B	6.5
	313	8.2	210	218.2	57.7	38	86	13		5	2B	9.9
	412	5.8	91.0	96.8	58.6	37	27	73		98	4A	6.9
	413	8.5	72.0	80.5	61.4	37	3	8		73	4B	7.8
	414	.6	74.0	74.6	57.1	37	30	6		60	4A	7.8
73	301	6.4	33.5	39.9	57.8	47	39	27		47	5A	9.4
	309	10.8	42.4	53.2	56.3	44	51	21		46	3A	8.4
	402	25.8	72.0	97.8	62.2	45	17	71		57	6A	9.9
	403	11.5	31.6	43.1	64.0	46	3	88		67	5B	9.2
Type B—Moderate Temperature, Open Window Rooms												
2 Bx.	301	3.1	35.7	38.8	70.7	25	25	8		0	4B	12.3
	302	31	38.4	69.4	70.7	25	16	4		48	3B	11.9
	316	16.4	46.5	62.9	69.7	24	29	9		100	5A	12.3
12	405	4.5	14.8	19.3	66.5	43	36	3		35	3B	13.6
	408	11.3	13.5	24.8	67.5	42	44	0		100	3B	13.1
	411	5.1	17.0	22.0	65.9	44	40	1		100	4A	13.8
22	415	3.7	7.4	11.1	69.9	45	9	8		44	5B	8.1
	416	8.5	6.5	15.0	69.7	48	5	30		23	4B	7.9
	418	8.0	0	8.0	69.3	43	13	3		0	5A	7.8
39	302	6.9	0	6.9	62.2	40	80	1		0	3B	14.9

TABLE XI (CONTINUED)

SCHOOL	ROOM	RESPIRATORY SICKNESS RATES			AV. TEMP.	AV. REL. HUMIDITY	PER CENT SESSIONS		PER CENT GIRLS	GRADE	SQ. FT. OF FLOOR SPACE PER PUPIL
		AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL			ESP. FRESH	ODOROUS			
39	308	21	.6	21.6	60.2	45	88	1	0	3A	13.4
	403	1.4	0	1.4	62.3	45	65	4	0	4A	12.7
59	318	15.1	60.9	76.0	64.8	36	5	6	56	2B	7.5
	411	10.9	90.6	101.5	65.7	32	12	13	100	5A	8.7
	416	1.5	14.6	16.1	65.6	32	4	12	7	4A	7.2
73	305	3.2	1.1	4.3	66.8	46	0	41	51	4A	11.1
	406	10.2	1.2	11.4	62.8	43	13	35	64	3B	12.0
	408	10.4	23.9	34.3	66.4	47	0	56	56	5A	9.5
165	302	10.4	21.3	31.7	69.4	30	21	3	0	5B	16.5
	306	5.7	37.9	43.6	67.7	33	26	3	0	4B	15.1
	309	26.4	47.5	73.9	71.8	27	6	21	0	4B	11.1
			Type C—Moderate Temperature, Fan Ventilated, Closed Window Rooms								
2 Bx.	207	13.0	64.2	77.2	69.5	30	26	18	0	3A	12.2
	307	.8	32.7	33.5	68.6	25	39	15	0	4A	18.6
	310	13.3	47.2	60.5	69.2	24	64	6	0	5A	13.8
22	402	7.0	4.1	11.1	68.4	42	11	3	100	4B	12.9
	403	10.5	12.0	22.5	67.4	46	14	1	100	4B	13.3
	404	8.9	25.4	34.3	68.5	43	14	3	100	4A	12.4
59	304	16.9	188	204.9	68.5	48	23	15	100	5A	14.0
	401	17.9	149	166.9	68.9	47	19	23	100	5A	15.2
	405	16.8	140	156.8	67.8	49	31	10	100	4B	14.2
	501	10.4	163	173.4	67.8	50	19	24	100	5B	14.0
	503	8.0	96.6	104.6	68.3	50	47	3	100	5B	13.5
	505	12.6	97.3	109.9	68.0	50	37	3	100	5B	14.5
147	210	8.4	45.8	54.2	66.4	38	3	28	41	3A	15.4
	304	1.5	72.5	74.0	68.0	36	9	47	47	4B	15.1
	305	9.6	21.5	31.1	67.5	36	8	26	39	5A	18.5
165	201	34.0	33.6	67.6	70.2	30	3	9	34	4A	15.3
	202	37.0	81.8	118.8	72.2	24	0	10	45	3B	19.6
	220	35.2	156.0	191.2	70.8	24	4	6	36	4A	16.8
	308	12.0	33.6	45.6	70.5	27	11	22	0	5B	12.0

TABLE XII

RECORDS OF INDIVIDUAL ROOMS IN SECOND STUDY

SCHOOL	ROOM	RESPIRATORY SICKNESS RATES PER CENT: AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL	AV. TEMP.	AV. REL. HUMIDITY	ESP. FRESH	ODOROUS	GRADE
Type A—Cold Open Window Rooms									
12	303	3.9	46.6	50.7	59.9	58.0	28	0	2 A
	409	8.1	13.7	21.8	61.3	54.4	18	0	4 B
	410	11.1	14.2	25.3	60.3	56.0	33	0	4 B
39	202	6.9	65.0	71.9	58.7	41.3	65	4	3 A
	203	13	40.0	53.0	56.2	43.4	72	2	4 A
	204	21.6	45.9	67.5	56.6	43.1	72	1	3 B
	205	6.7	30.3	37.0	58.7	42.2	46	7	3 B
	206	9.3	24.9	34.2	58.3	40.3	55	0	3 A
	207	11.7	18.4	30.1	57.6	41.4	60	2	4 B
59	308	4.0	165	169	60.0	48.0	100	0	3 B
	311	8.5	144.	152.5	58.5	48.9	100	0	3 A
	313	26.8	213	239.8	59.0	44.7	85	11	2 A
	412	4.3	150	154.3	58.1	45.1	100	0	4 A
	413	9.2	147	156.2	60.0	48.0	98	2	4 B
	414	3.0	124	127	57.8	48.4	100	0	4 A
	416	8.0	91.3	99.3	57.6	46.5	100	0	4 B
73	301	5.1	44.1	49.2	60.2	44.8	71	2	5 B
	309	11.6	34.8	46.4	58.9	45.9	67	2	4 A
	402	6.1	18.4	24.5	60.5	44.7	72	5	6 A
	403	6.2	39.4	45.6	63.5	45.3	50	29	5 B
Type B—Moderate Temperature, Open Window Rooms									
2 Bx.	301	10.7	9.7	20.4	68.6	38.5	0	13	2 B
	302	7.7	5.8	13.5	69.0	38.5	0	23	2 B
	316	11.6	12.2	23.8	65.4	40.0	0	24	5 A
12	405	9.5	33.2	42.7	62.2	57.8	6	1	4 A
	408	15.9	31.4	47.3	63.5	59.0	1	2	3 B
12	411	9.9	5.8	15.7	63.8	60.6	3	10	4 A
22	415	0	0	0	67.6	57.7	17	82	5 B
	416	5.5	2.7	8.2	68.2	56.0	23	72	6 A
	418	.9	24.6	25.5	67.3	56.7	29	65	5 B
33 Bx.	13	21.4	55.7	77.1	69.3	31.3	0	9	8 B
	23	27.5	47.5	75.0	69.4	37.2	1	21	7 A
39	302	10.9	33.6	44.5	63.0	40.3	19	0	4 A
	308	8.6	42.8	51.4	65.0	37.7	23	3	5 A
	408	2.9	58.0	60.9	65.2	39.0	9	31	5 A
59	318	3.7	187	190.7	64.9	48.5	4	61	3 A
	415	16.2	158	174.2	58.8	48.2	100	0	4 B
73	305	9.7	23	32.7	61.6	46.5	45	10	4 B
	406	2.7	41	43.7	67.1	43.3	17	35	3 A
	408	16	51	67	61.1	43.6	74	4	3 B
97	302	6.4	37	43	67.0	38.2	17	62	5 B
	313	7.1	50	57	67.3	41.8	71	17	4 A
	501	3.0	55	58	65.9	44.8	59	18	6 B
115	312	23.3	—	—	67.8	28.8	3	3	4 B
	502	40.2	—	—	67.9	26.2	2	3	5 B

TABLE XII (CONTINUED)

SCHOOL	ROOM	RESPIRATORY SICKNESS RATES: AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL	AV. TEMP.	AV. REL. HUMIDITY	PER CENT SESSIONS: ESP. FRESH	ODOROUS	GRADE
	503	29.8	—	—	67.0	29.5	0	3	5 B
165	302	1.3	99	100.3	67.2	35.9	0	0	5 B
	306	10.4	56	66.4	66.8	35.7	0	0	4 B
	309	8.1	33	41.1	68.9	36.1	0	0	4 B
	Type C—Moderate Temp., Fan Ventilated, Closed Window Rooms								
2 Bx.	207	22.4	25.5	47.9	66.1	39.6	0	13	2 A
	307	13.2	17.0	30.2	65.8	41.2	0	35	3 B
	310	1.3	3.8	5.1	66.5	41.5	0	20	4 B
22	402	4.8	5.6	10.4	66.5	57.0	75	16	6 A
	403	3.5	1.3	4.8	65.8	56.2	77	23	4 B
22	404	14.4	18	32.4	66.5	54.9	66	17	4 B
33 Bx.	14	29.3	23	52.3	71.0	28.6	1	2	6 B
	24	45.4	125	170.4	70.2	32.0	3	3	5 A
51 Bx.	203(Hum.)	4.0	148	152	67.6	43.6	0	39	6 A
	205	14.7	50	64.7	67.4	29.0	0	2	6 A
59	203	16.8	182	198.8	69.2	47	10	12	6 B
	205	13.9	275	288.9	69.2	45.5	17	5	6 B
	206	9.9	316	325.9	69.6	46	16	1	6 A
	501	12.4	256	268.4	68.9	49	7	6	5 B
	503	9.1	197	206.1	69.2	47.9	25	1	5 B
	505	8.3	112	120.3	68.7	49.3	2	13	6 A
97	303	2.3	34.1	36.4	68.0	43.1	84	2	4 B
	308	2.7	34.7	37.4	68.3	40.2	79	4	5 A
	502	6.8	43.3	50.1	66.3	44.8	91	3	6 B
115	308	44	24.8	68.8	67	27.2	2	5	4 A
	311	50.4	51.6	102	68.2	25.2	0	6	4 B
147	210	16.6	11.9	28.5	68.1	42.4	30	13	3 A
	304	3.6	6.8	10.4	67.7	40.4	22	36	4 B
	305	4.2	12.4	16.6	67.1	42	49	9	5 A
165	201	11.9	196	207.9	68.4	33.3	0	0	4 A
	202	5.3	151	156.3	68.0	34.5	0	0	3 B
	220	10	181	191	69.2	35	0	0	4 A
	308	1.8	154	155.8	68.7	35	0	0	5 B

the degree desired. If the pupils in one district are by reason of hereditary and environmental influences more susceptible to colds, then this school will unduly raise the sickness rate in the ventilation type within which the majority of its records fall.

Of the 12 schools used in the two studies, only one possessed all three examples of ventilation. In one other instance the three types were represented by two schools a block or so apart, one school having Type C and the other, Types A and B. In all other instances there were not more than two types represented within a school building, some buildings having A and B and others, B and C. The division of rooms is revealed in the table below.

One can readily appreciate by looking at the table how the results would be affected if, say, School 39 were given to very little sickness and School 147 to

TABLE XIII

DISTRIBUTION OF TEST ROOMS AMONG THE TWELVE SCHOOLS BY VENTILATION TYPE

SCHOOL	FIRST STUDY			SECOND STUDY		
	A	*B*	*C*	*A*	*B*	*C*
12	3	3	0	3	3	0
147	0	0	3	0	0	3
22	0	3	3	0	3	3
59	5	3	6	7	2	6
73	4	3	0	4	3	0
165	0	3	4	0	3	4
39	6	3	0	6	3	0
2 Bx.	0	3	3	0	3	3
33 Bx.				0	2	2
51 Bx.				0	0	2
97				0	3	3
115				0	3	2
Total	18	21	19	20	28	28

a great deal. In the summary of all rooms Type A, having 6 rooms in School 39 would have a low sickness rate, not because of ventilation, but because of its personnel, and Type C would be inclined to have a high rate, not because of ventilation but because of the numbers of children from School 147. Theoretically this influence should have been eliminated at the beginning, but actually this was found impossible.

The total respiratory illness rate including both absentees and those present in each school is shown in Table XIV.

TABLE XIV

TOTAL RESPIRATORY ILLNESS RATES BY SCHOOLS

SCHOOL	FIRST STUDY	SECOND STUDY
97	—	46.7
22	100.3	13.6
12 & 147	31.1	27.9
73	37.4	44.2
51 Bx.	—	106.4
59	103.1	193.3
39	12.6	51.6
2 Bx.	55.9	24.2
165	77.9	123.3
33 Bx.	—	92.6

A considerable variation is seen in the illness rates. Schools 59 and 165 are relatively high in both studies. Schools 12, 147 and 73 are low in both. School 22 is high in the first study and extremely low in the second.

We may examine the effect of ventilation apart from these extraneous influences mentioned first by inspecting the records of each school by itself and secondly by balancing the influence which each school exerts on the total.

In an effort to illustrate the comparison of illness rates within each school we have prepared Charts I and II, the former showing Types A and B and the latter, B and C. Both measures of illness among the absent and among those present are included. The frequency with which one ventilation type exceeds the other in amount of illness conveys an impression that is not brought out in the averages for each ventilation type.

In the comparison of the window ventilated rooms from the chart, Type B exceeds Type A in respiratory illness in nine instances. In the remaining seven instances Type A exceeds Type B. There is then no prevailing superiority of one type over another. It will be noted in the summary at the bottom of the chart, where the rates have been averaged, that Type B shows less re-

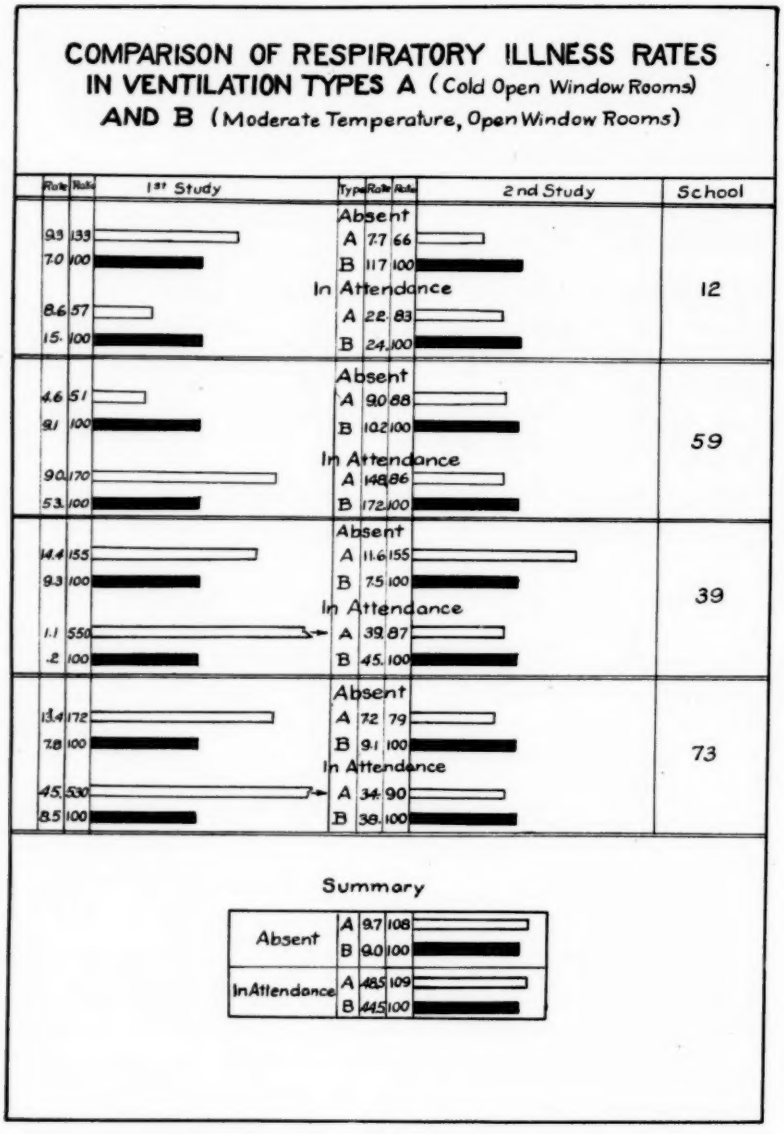

Chart I.

spiratory illness than A. This is due to the influence of several high rates in Type A. Incidentally this illustrates how an erroneous impression may be gained from averages alone.

In the comparison between Types B and C, the latter exceeds the former in respiratory illness in eighteen instances; whereas B exceeds C in only seven

instances. This result is much more significant than in the previous comparison. The averages of the rates are consistent with the tendency of the individual instances. With the new schools used in the second study included, the average for Type C exceeds B in both measures of respiratory illness. The same is true with the new schools omitted.

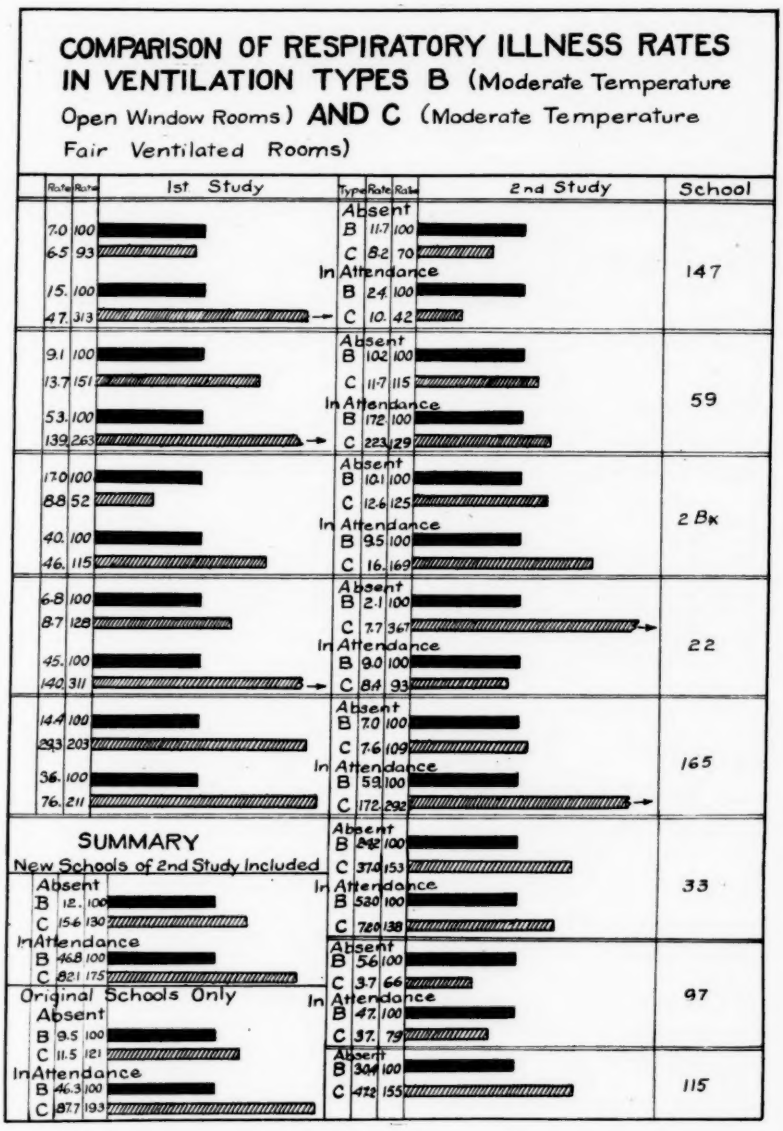

Chart II.

This analysis confirms what has been brought out previously—that the difference between Types A and B is insignificant; whereas, between B and C there is a distinct excess of illness in the fan ventilated rooms.

In School 59 all three types of ventilation are represented. We have in this instance a good measure of ventilation influences on pupils of the same

general characteristics. The fan ventilated rooms have the greatest respiratory illness in both studies. The relative positions of A and B are not the same in the two studies, the cold rooms having more illness in the first study and less in the second.

TABLE XV

RESPIRATORY ILLNESS RATES IN THREE VENTILATION TYPES AT SCHOOL 59

VENTILATION TYPE	FIRST STUDY			SECOND STUDY		
	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM
A	4.6	90	94.6	9.0	148	157
B	9.1	53	62.1	10.2	172	182
C	13.7	139	152.7	11.7	223	235

The room temperature for each type averaged as follows:

A	58.8	58.7
B	65.4	61.9
C	68.2	69.1

The fan ventilated rooms were the warmest, exceeding the Type B rooms by 2.8 degrees in the first study and by 7.2 degrees in the second. These results are consistent in showing less illness in the window ventilated rooms.

In one other instance the three ventilation types are to be found divided between two schools within a block of each other and for all practical purposes the characteristics of the pupils are the same. The sickness rates for Schools 12 and 147 are shown in Table XVI.

TABLE XVI

RESPIRATORY ILLNESS RATES IN THREE VENTILATION TYPES AT SCHOOLS 12 AND 147

VENTILATION TYPE	FIRST STUDY			SECOND STUDY		
	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM
A	9.3	8.6	17.9	7.7	22	29.7
B	7.0	15	22.0	11.7	24	35.7
C	6.5	47	53.5	8.2	10	18

(Temperature—1st Study A 60.5, B 66.6, C 67.3; 2nd Study, A 60.5, B 63.2, C 67.6.)

The fan ventilated rooms appear to better advantage in this instance, for illness is lowest in Type C in the second study. In the first study the total illness is greatest in the fan ventilated rooms, although the illness causing absence is the lowest of the three types.

The Type C rooms at School 59 were unusually well equipped with mechanical ventilation facilities. The air is humidified before entering the room, and the blowers are capably managed. The fan equipment at School 147 is older and the rooms were not thoroughly aerated at all times, and windows were frequently found open.

In view of the oft repeated assertion that humidification and air washing in combination with the plenum fan is from many standpoints a superior

form of ventilation, the figures of this study are of special interest. A modern form of mechanical ventilation with warm temperature is associated with more respiratory sickness than naturally ventilated rooms with gravity exhaust. On the other hand, fan ventilation, lacking many modern features, as in P. S. 147, is associated with less respiratory illness than naturally ventilated rooms. At P. S. 147 the temperature of the fan rooms was lower than at P. S. 59. Raising the temperature over 68 degrees would seem to be more disturbing to health than reducing the volume of air passing through the rooms.

The temperature of the A rooms in all instances was lower than those selected as B rooms. In general the C rooms were warmer than B, although there were some exceptions to this.

TABLE XVII

AVERAGE TEMPERATURE AND TOTAL RESPIRATORY ILLNESS RATES BY VENTILATION TYPES IN EACH SCHOOL

SCHOOL	VENTILATION TYPE	FIRST STUDY		SECOND STUDY	
		TEMP.	RATE	TEMP.	RATE
12	A	60.5	17.9	60.5	29.7
	B	66.6	22.0	63.2	35.7
147	C	67.3	53.5	67.6	18.2
39	A	57.2	15.5	57.7	50.6
	B	61.6	9.5	64.4	52.5
59	A	58.8	94.6	58.7	157
	B	65.4	62.1	61.9	182
	C	68.2	153	69.1	235
73	A	60.1	58.4	60.8	41.2
	B	65.3	16.3	66.6	47.1
2 Bx.	B	70.4	57.0	67.7	19.6
	C	69.1	54.8	66.1	28.6
22	B	69.6	51.8	67.7	11.1
	C	68.1	22.7	66.3	16.1
165	B	69.6	50.4	67.6	66.0
	C	70.9	105	68.6	186
33 Bx.	B	—		69.4	76.2
	C	—		70.6	109
115	B	—		67.6	30.4*
	C	"		67.6	47.2*
97	B			66.7	52.6
	C			67.6	40.7

*This is absence illness only.

Whenever temperatures are over 68 degrees, the warmer rooms have the greater sickness, regardless of whether Type B or C. When temperatures are below 68, the window rooms have less sickness in four instances and more sickness in two instances.

It may be pointed out in this connection that even where the temperatures of a fan and window room, as measured at the three-foot level, are identical, the window room is actually cooler, for the temperature at the floor level is always lower in the window rooms.

The second means of shedding light on the significance of the grand averages is by equalizing the influence of each school in each ventilation group. In doing this we have combined the two studies, omitting the schools that were not represented in both. Where there are 9 rooms at a school, 6 in Type A and 3 in Type B, we have reduced the number in the first type to three by averaging the two highest readings, the two lowest and the two intermediate. Where there are four readings, we have averaged the two highest and used the other two as they stand.*

We have illustrated in Tables XVIII and XIX the manner of making this computation by showing the selected rates used along with the original figures.

In Table XX will be found the averages of the rates both actual and ad-

TABLE XVIII

ACTUAL AND SELECTED RESPIRATORY ILLNESS RATES BY ROOMS
COMPARISON OF TYPES A AND B (BOTH STUDIES COMBINED)

	ABSENCE RATE				ILLNESS IN ATTENDANCE RATE			
	TYPE A		TYPE B		TYPE A		TYPE B	
SCHOOL	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED
12	15.	15.	4.5	4.5	10.	10.	15.	15.
	8.6	8.6	11.3	11.3	3.	3.	14	14.
	5.0	5.0	5.1	5.1	16.	16.	17.	17.
	3.9	3.9	9.5	9.5	47.	47.	33.	33.
	8.1	8.1	15.9	15.9	14.	14.	31.	31.
	11.1	11.1	9.9	9.9	14.	14.	6.	6.
39	11.	11.	6.9	6.9	2.	2.	0.	0.
	11.		21.	21.	2.			
	14.	14.	1.4	1.4	2.	1.	1.	1.
	14				.6			
	21	19.			0	0	0	0
	17				0			
	6.9	6.8	10.9	10.9	65.	55.	34.	34.
	6.7		8.6	8.6	46			
	9.3	10.5	2.9	2.9	40	35.	43.	43.
	11.7				30			
	13.0	17.3			25.	21.	58.	58.
	21.6				18.			
59	.6	.6	15.1	15.1	210.	151.	61.	61.
	.6		10.9	10.9	91			
	8.2	8.4	1.5	1.5	72.	47.	91.	91.
	8.5				21.			
	5.8	5.8			74.	74.	15.	15.
	26.8		3.7	3.7	213.			
	9.2	18.	16.2	16.2	165	189.	187.	187.
	8.5			10.	124			
	8.	8.3			91	108	158.	158.
	4.3				144			
	3.0	3.8			150.	147.		173.
	4.				147.			
73	25.8	18.8	3.2	3.2	57.	57.	1.	1.
	11.5		10.2	10.2	34.	34.	1.	1.
	6.4	6.4	10.4	10.4	32.	32.	24.	24.
	10.8	10.8						
	11.6	8.9	9.7	9.7	42.	42.	23.	23.
	6.2		2.7	2.7	35.	35.	41.	41.
	6.1	6.1	16.	16.	18.	18.	51.	51.
	5.1	5.1						

*This might be done by averaging the two lowest or the two intermediate rates, letting the highest value stand. The difference, however, is too slight to alter the final result.

TABLE XIX

ACTUAL AND SELECTED RESPIRATORY ILLNESS RATES BY ROOMS
COMPARISON OF TYPES B AND C (BOTH STUDIES COMBINED)

	ABSENCE RATES				ILLNESS IN ATTENDANCE RATES			
	TYPE B		TYPE C		TYPE B		TYPE C	
SCHOOL	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED
2 Bx.	3.1	3.1	13.	13.	36.	36.	64.	64.
	31.	31.	.8	.8	38.	38.	33.	33.
	16.4	16.4	13.3	13.3	47.	47.	47.	47.
	10.7	10.7	22.4	22.4	10.	10.	26	26.
	7.7	7.7	13.2	13.2	6.	6.	17.	17.
	11.6	11.6	1.3	1.3	12.	12.	4.	4.
22	5.7	5.7	7.0	7.0	7.	7.	4.	4.
	8.5	8.5	10.5	10.5	7.	7.	12	12.
	8.0	8.0	8.9	8.9	0	0	25.	25.
	0	0	4.8	4.8	0	0	6.	6.
	5.5	5.5	3.5	3.5	3.	3.	1.	1.
	.9	.9	14.4	14.4	25.	25.	18.	18.
59	15.1	15.1	17.9	17.4	61.	61.	188.	176
	10.9	10.9	16.9		91.	91.	163.	
	1.5	1.5	16.8	14.7	15.	15.	149.	145.
			12.6				140.	
			10.4	9.2			97.	97.
			8.0				97.	
	3.7	3.7	16.8	15.4	187.	187.	316	296.
	16.2	16.2	13.9		158.	158.	275.	
		10.	12.4	11.2		173.	256.	227.
			9.9				197.	
			9.1	8.7			182	147.
			8.3				112	
12 &	4.5	4.5	8.4	8.4	15.	15.	46.	46.
147	11.3	11.3	1.5	1.5	14.	14.	73.	73.
	5.1	5.1	9.6	9.6	17.	17.	22.	22.
	9.5	9.5	16.6	16.6	33.	33.	12.	12.
	15.9	15.9	3.6	3.6	31.	31.	7.	7.
	9.9	9.9	4.2	4.2	6.	6.	12.	12.
165	10.4	10.4	37.0	36.1	21.	21.	156.	119.
	5.7	5.7	35.0		38.	38.	82.	
	26.4	26.4	34	34.	48.	48.	34.	34.
			12.	12.			34.	34.
	1.3	1.3	11.9	11.	99.	99.	196.	189.
	10.4	10.4	10.		56.	56.	181.	
	8.1	8.1	5.3	5.3	33.	33.	154.	154.
			1.8	1.8			151.	151.

justed. Where the school influence is thus equalized in each ventilation type the average illness rates are appreciably different from the uncorrected averages. In the comparison of Types A and B only those schools have been used which possessed both A and B rooms. A B room in a school not having

TABLE XX

COMPARATIVE RATES OF RESPIRATORY ILLNESS WITH UNCORRECTED AND BALANCED AVERAGES
(BOTH STUDIES COMBINED)

VENTILATION TYPE	ABSENCE RATE		ILLNESS IN ATTENDANCE RATE	
	UNCORRECTED	BALANCED	UNCORRECTED	BALANCED
A	10.0	9.6	57	48
B	9.0	9.1	39	45
B	9.4	9.4	38	43
C	12.0	11.1	94	73

an A room is omitted. Similarly, in the comparisons of B and C rooms, B rooms are omitted where there is lacking a C room in the same school.

Before correcting for number of rooms the absence rates for A and B were 10.0 and 9.0. After eliminating the abnormal influence of the schools with the most rooms, the rates are 9.6 and 9.1. In the first instance Type A was greater than B, largely because of the greater number of rooms at Schools 39 and 59, where the rates are higher.

The effect of eliminating the school influence is even more noticeable with the rates for illness in attendance. Without correction the rate for A was 57 and for B, 39, an appreciable difference. When the influence of each school has been equalized, the rates are 48 for A and 45 for B. In the former instance A exceeds B only because it possessed more rooms at P. S. 59, where the rates are high, and not because of any ventilation influence.

The corrected absence rates for Types B and C are 9.4 and 11.1, a difference of 1.7. Before the correction had been applied the rates were 9.4 and 12.0, a difference of 2.6. It was the greater number of rooms at P. S. 59, where the rates are high, that raised the average for Type C. When this influence is modified the difference between the two types is less.

A marked alteration is also produced in the rates for illness-in-attendance. Without the correction for number of rooms, the figure for B is 38 and for C, 94, a difference of 56. Eliminating the school influence the rates are 43 and 73, a difference of only 30. School 59 is largely to blame for the apparent wide difference in the types. With an equal number of rooms at each school we obtain a truer conception of the difference in ventilation types.

Having arrived at comparative figures which are believed to give a fairly accurate measure of the ventilation influence, we are confronted with the interpretation of these results. Is the difference of 0.5 between the absence rate in A and B significant, or is it a chance result which, if the experiment were repeated, would reverse itself? We can answer this question by determining the probable error of the averages. If the differences are statistically significant, they will represent at least three times the value of the probable error. If the differences are no greater than the probable error, then we cannot say that ventilation exercises an unmistakable effect on the health of these school children.

The formula for the probable error is

$$\text{P. E.} = 0.6745 \sqrt{\frac{\Sigma X^2}{n}}$$

The computation of the probable error is made by averaging the absence rates in each type: finding the deviation of each rate from the average; squaring each deviation; averaging these squares; determining the square root from this average; dividing this figure by the square root of the number of cases, which gives the standard deviation and finally multiplying this figure by the constant, 0.6745, which gives the probable error, or P. E.

The difference in the absence rates between Types A and B, 0.5, is less than the probable error, or approximately 0.7, and in consequence, is without significance. This means that the evidence is insufficient to prove that either

type of ventilation is superior to the other in so far as respiratory illness is concerned.

Respiratory illness of a less severe nature and sufficient to keep children out of school is likewise, judging by the fact that the probable error exceeds the difference in rates, no different in a cold, window ventilated room with a temperature around 59 degrees than in a cool, window ventilated room whose temperature is in the neighborhood of 64 degrees. This finding is in agreement with the original computations for the entire group of rooms.

The differences between Types B and C are more marked. The higher probable error for absence rates, that is for Type C, is 1.0. The difference

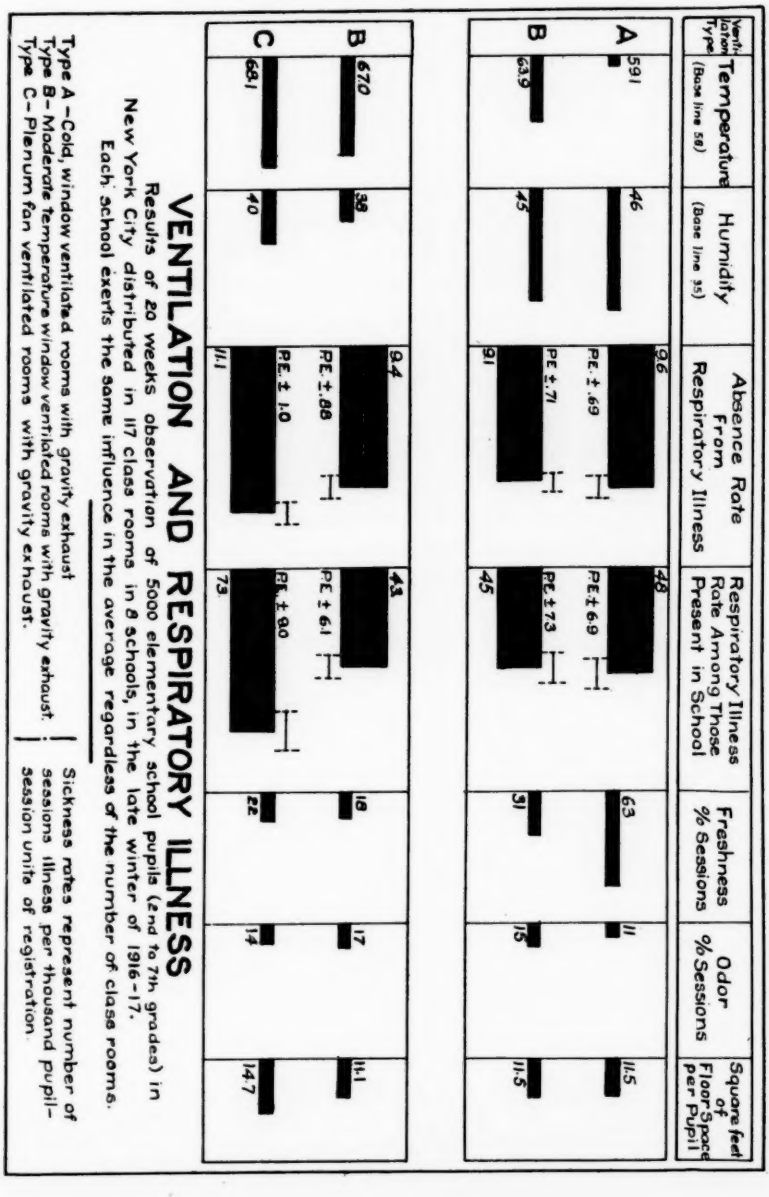
VENTILATION AND RESPIRATORY ILLNESS
Results of 20 weeks observation of 5000 elementary school pupils (2nd to 7th grades) in New York City distributed in 117 class rooms in 8 schools, in the late winter of 1916-17.
Each school exerts the same influence in the average regardless of the number of class rooms.
Type A – Cold, window ventilated rooms with gravity exhaust
Type B – Moderate temperature window ventilated rooms with gravity exhaust.
Type C – Plenum fan ventilated rooms with gravity exhaust.
Sickness rates represent number of sessions illness per thousand pupil-session units of registration.
Ventilation Type
Temperature (Base line 58)
Humidity (Base line 35)
Absence Rate From Respiratory Illness
Respiratory Illness Rate Among Those Present in School
Freshness % Sessions
Odor % Sessions
Square feet of Floor Space per Pupil
A 591 46 96 PE. ± .69 48 PE ± 69 63 11 11.5
B 639 45 91 PE. ± .71 45 PE ± 73 31 15 11.5
B 670 39 94 PE ± .86 43 PE ± 61 18 17 11.1
C 681 40 111 PE ± 1.0 73 PE ± 90 22 14 14.7

Chart III.

TABLE XXI
PROBABLE ERROR OF RESPIRATORY SICKNESS RATES
(BOTH STUDIES COMBINED)

	DIFFERENCE BETWEEN VENTILATION TYPES A AND B	PROBABLE ERROR	DIFFERENCE BETWEEN VENTILATION TYPES B AND C	PROBABLE ERROR
Absence Rate	0.5	A ± .69 B ± .71	1.7	A ± .88 B ± 1.00
Sickness in Attendance Rate	3.0	A ± 6.9 B ± 7.3	30	A ± 6.1 B ± 9.0

(Note: Only schools used in both studies are included in the above.)

between the sickness rates is 1.7. While this difference is not greater than three times the probable error, the mere fact that it is greater suggests at least a tendency for Type C rooms to be more conducive to respiratory illness than those of Type B.

Minor respiratory illness insufficient to cause absence amounts to a rate of 43 in Type B rooms and to 73 in Type C. This is a difference of 30. The greater probable error is 9.0. The difference is thus more than three times the probable error and statistically may be regarded as significant. Interpreted in other words the system of fan ventilation representing Type C is more conducive to respiratory illness among school children than the window ventilation methods of Type B.

In Chart III are represented graphically the absence rates as well as other facts pertinent to the matter. The difference in average temperature between Type A and B rooms was 4.8 degrees. Relative humidity was almost identical, being 46 per cent in the A rooms and 45 in B. The A rooms were judged exceptionally fresh 63 per cent of the time as against 31 per cent for B. The air possessed a noticeable odor 11 per cent of the time in A and 15 per cent in B. The degree of congestion was the same in both types, namely: 11.5 square feet of floor space per pupil.

In the second comparison, the Type B rooms averaged 67.0 degrees temperature as against 68.1 for the C rooms, a difference of but 1.1 degrees. Relative humidity was 38 per cent in B and 40 per cent in C. The B rooms were judged exceptionally fresh 18 per cent of the time, the C rooms 22 per cent. Odor was noticeable 17 per cent of the sessions in B and only 14 in C. The B rooms were appreciably more congested, the square feet of floor space per pupil being 11.1 as against 14.7 in the C rooms.

The following conclusions appear justified from the evidence:

1. Respiratory sickness is no greater in a window ventilated schoolroom kept around 59 degrees than it is in a room where temperature is 64.

2. Respiratory sickness is greater in fan ventilated rooms, such as are represented in this study, than in window ventilated rooms, even though there is not more than a degree difference in temperature, and the fan rooms are more spacious.

3. It is low temperature rather than chemical purity of the air which conveys the sensation of freshness.

(To be concluded)

LABORATORY METHODS

A COLORIMETRIC METHOD FOR THE ESTIMATION OF MORPHINE IN COLLOIDAL MIXTURES AND TISSUES*

By Harry Gauss, M.D., Denver, Colo.

THE necessity for a rapid, accurate method of analysis for morphine frequently arises in toxicological, as well as in work in experimental therapy. It was during the course of investigations on the effect of morphine upon experimental tuberculosis that a survey of the literature revealed the necessity of further study to develop such a method. It was rather noteworthy that in spite of the many color reactions for morphine that are available, which are mostly evanescent and unstable, there was found no suitable colorimetric method applicable to a rapid and accurate determination of this alkaloid in tissues.

By elimination from a large number of color reactions for morphine, two were chosen as being the most suitable for the purpose at hand, the one the reaction with Marquis' reagent (a mixture of one part of pure 40 per cent formaldehyde in 15 parts of concentrated sulphuric acid C. P.) giving a purple-red color with morphine which gradually but slowly changes to violet and then to a fairly permanent blue; and the other the reaction with Lafon's reagent (a solution of a selenite or selenic acid 0.5 per cent in concentrated sulphuric acid C. P.) giving an evanescent blue to intense blue with this alkaloid and finally a fairly persistent olive green. The latter reagent has recently been recommended by Morgulis and Levine[1] and has been used not as a quantitative colorimetric method but as an extinction point method giving only relatively quantitative results. The reagent was found by them to be sensitive to 0.025 milligram morphine sulphate with a positive reaction, while they could still recognize 0.0125 milligram of the pure alkaloid salt. When mixed with tissues no less than 0.5 milligram of morphine sulphate (0.2 mg. of the alkaloid) could be detected. Their method of extraction was to heat the tissues with 2 per cent tartaric acid, in proportion of 20 grams of substance to 100 c.c. of reagent heating for 30 minutes on the water-bath, cooling and straining through cheese cloth, uniting the collected washings after the last is free from acid, evaporating to a thick consistency, adding sodium bicarbonate until effervescence ceases and then adding a slight excess. The alkaloid thus being set free is extracted from the dried ground mass by means of chloroform using 50 c.c. for the extraction. Unfortunately the margin of error of their method is entirely too great to give more than approximate results.

*From the Research Department, National Jewish Hospital for Consumptives, Denver, Colorado.

Heidreschka and Faul[2] have used the Marquis' reagent for colorimetric purposes and have found it sensitive quantitatively in concentrations varying from 1 in 500 to 1 in 25,000. The colors are examined by transmitted light, since in reflected light an actual color change from blue to bluish brown renders the comparison untrustworthy. Their determinations were made on poppies. They also used a colorimetric method described by Georges and Gascard[3] in which a yellow coloration is obtained by means of iodic acid added to solutions of morphine in dilute one tenth normal hydrochloric acid. Quantitative observations with a Dubosecq colorimeter using this color test can only be made at concentrations between 1 in 500 and 1 in 5000 with this reagent.

Colorimetric methods are convenient on account of the rapidity with which analyses can be made, the fair accuracy attainable and the ease of comparison with a standard of a pure sample of the substance being analyzed for. Most colorimetric methods depend upon the formation of a stable colored compound but are applicable to certain unstable colors provided they change only slowly.

During the course of investigations, necessity occurred for a fairly accurate rapid quantitative method of extraction of morphine from colloidal mixtures (such as agar) and tissues and subsequent rapid determination. The usually described methods proved cumbersome and many were totally inadequate. It was for these reasons that the following detailed investigations were carried on. Unfortunately no stable color is developed by morphine as far as could be found in the literature but the most suitable color was found to occur with Marquis' reagent and this color is evanescent. This, however, necessitated the use of a standard of known amount of morphine to which the reagent was added simultaneously to a preparation of the unknown. Since, however, the color changes occur with equal speed especially with approximately equal amounts of morphine, this proved entirely satisfactory. It also proved to be quite flexible as is shown by the following experiment.

TABLE I

THE COLOR REACTION OF MORPHINE SULPHATE WITH MARQUIS' REAGENT IN PROPORTION TO THE AMOUNT OF MORPHINE BY WEIGHT

MORPHINE SULPHATE BY WEIGHT		COLORIMETER READINGS	
Tube I	Tube II	Tube I	Tube II
1 mg.	1 mg.	10.0	10.0
1 mg.	2 mg.	10.0	5.0
1 mg.	3 mg.	10.0	3.3
1 mg.	4 mg.	10.0	2.5
1 mg.	5 mg.	10.0	2.0

Solutions containing 1, 2, 3, 4 and 5 milligrams of morphine sulphate were placed in separate beakers and evaporated to dryness on the water-bath. To each of two beakers was added simultaneously 10 cubic centimeters Marquis' reagent and the colors dissolved by stirring with a glass rod. An assistant is employed to mix the color in one of the beakers to insure a simul-

taneous reaction. It was thus found that the depth of color varied directly with the proportion of morphine sulphate as is indicated in Table I. Color comparison is inversely proportional to the amount of morphine sulphate by weight.

The Marquis' reagent used in these experiments was always prepared fresh just before use from C. P. reagents—formaldehyde solution and sulphuric acid. The reagent must be perfectly colorless. The amount of Marquis' reagent used in each test was 10 c.c. In control tests using from 1.0 milligram to 0.005 milligram of morphine sulphate, it was found that 0.05 milligram could be determined with reasonable accuracy in the colorimeter* as is shown in Table II.

TABLE II

THE LIMIT OF DELICACY OF THE COLORIMETRIC TEST FOR MORPHINE SULPHATE

MORPHINE SULPHATE BY WEIGHT		COLORIMETRIC READING (WITH UNIT VOLUME DILUTION—10 C.C.)	
Tube I	Tube II	Tube I	Tube II
1 mg.	0.50 mg.	10.0	20.0
1 mg.	0.25 mg.	5.0	20.0
0.2 mg.	0.10 mg.	10.0	20.0
0.1 mg.	0.05 mg.	10.0	20.0

Since the extinction point with the Marquis' reagent is far below the quantitative colorimetric range as determined in a colorimeter a study of this phase seemed desirable. For comparison the extinction point of morphine sulphate with Lafon's reagent (selenium-sulphuric acid) was also determined since at times it is desirable in determining small amounts of morphine to use two tests. The results of the study of the extinction point for Marquis' and Lafon's reagents are given in Table III.

The comparison of the extinction points of morphine sulphate with Mar-

TABLE III

THE EXTINCTION POINT OF MORPHINE SULPHATE WITH MARQUIS' AND LAFON'S REAGENTS

AMOUNTS OF MORPHINE SULPHATE TESTED BY WEIGHT	RESULTS	
	WITH MARQUIS' REAGENT ($HCOH$ H_2SO_4)	WITH LAFON'S REAGENT ($H_2S_2O_3$ H_2SO_4)
0.025 mg.	++*	++
0.010 "	++	++
0.009 "	++	++
0.008 "	++	++ definite green blue color
0.007 "	++ definite red color	+
0.006 "	+	±
0.005 "	+	+
0.004 "	+ less marked red color	+ still a slight green blue color
0.003 "	+ still visibly red	± doubtful reaction
0.002 "	± doubtful reaction	−
0.001 "	−	−

*The marking −, ±, + and ++ indicate the intensity of the color reaction obtained with 1 c.c. of the reagent—usually in the extinction method the color appears only at the site of the morphine and not diffused throughout the reagent. These results are the average of three readings on the same amount of morphine sulphate.

*Throughout this entire investigation the Kober Colorimeter-Nephelometer was used.

quis' and Lafon's reagent reveal very little difference in the sensitiveness of the two reagents although the Marquis' reagent is slightly more delicate.

In order to further test the colorimetric method for morphine sulphate using the color developed with the Marquis' reagent some commercial one-eighth grain hypodermic tablets of morphine sulphate obtained from two different firms were analyzed as follows: A tablet was pulverized in a beaker, to it was added 50 cubic centimeters of fresh Marquis' reagent, the mixture being well stirred to obtain a uniform color. This solution was then compared to a standard made by dissolving one milligram of chemically pure morphine sulphate in 10 cubic centimeters of Marquis' reagent. The depths of color were compared in the Kober colorimeter, with the final computed contents of the tablets (3 of each make) as given in Table IV.

TABLE IV

THE COLORIMETRIC ANALYSIS OF ONE-EIGHTH GRAIN HYPODERMIC TABLETS OF MORPHINE SULPHATE BY THE USE OF MARQUIS' REAGENT

TESTS	TABLETS PREPARED BY FIRM IN INDIANAPOLIS	BALTIMORE
1	7.77 milligrams*	7.56 milligrams
2	7.46 milligrams	7.46 milligrams
3	7.62 milligrams	7.93 milligrams

*These tablets should theoretically contain 8.1 milligrams of morphine sulphate.

Thus far our analyses have been for the pure alkaloid (combined with inorganic radicals or mixed with other inorganic salts) not mixed with organic colloidal matter or tissues. When present in the latter, recourse must first be had to methods of extraction. Since the extraction from agar-agar was of interest to us in certain chemotherapy studies, extraction from this colloid was first studied. It also seemed that with slight modification a method applicable to agar could easily be used for tissues. Most methods of extraction of morphine from organic material contemplate first forming the alkaloidal salt, obtaining it in aqueous solution and finally extracting the pure alkaloid by means of an alkaloid solvent such as chloroform, after the salt has been decomposed by the addition of an appropriate alkali.

As a result of some preliminary tests it appeared that the crucial point in the quantitative recovery is the extraction with chloroform and for this reason several modifications of chloroform extraction were tested.

Four ten cubic centimeter portions of a solution, each containing 5 milligrams of morphine sulphate, were used. Two of these were alkalinized with ammonium hydroxide and two with sodium bicarbonate, one of each of these two sets was extracted with cold chloroform and the other with hot chloroform—each extraction being made with 10 cubic centimeters of chloroform. The chloroform extracts were all separated in a separatory funnel and each extraction analyzed for its morphine sulphate content. The results of the individual extractions and number necessary to completely extract the 5 milligrams of morphine sulphate are given in Table V.

TABLE V

AN ANALYSIS OF THE CHLOROFORM EXTRACTIONS NECESSARY TO RECOVER 5 MILLIGRAMS OF MORPHINE SULPHATE FROM AQUEOUS SOLUTION UNDER CERTAIN CONDITIONS

	ALKALIZED WITH			
	AMMONIUM HYDROXIDE		SODIUM BICARBONATE	
NUMBER OF EXTRACTION	EXTRACTED WITH COLD CH Cl_3	EXTRACTED WITH HOT CH Cl_3	EXTRACTED WITH COLD CH Cl_3	EXTRACTED WITH HOT CH Cl_3
1	0.115	0.220	1.666	2.500
2	0.356	0.243	1.052	1.818
3	0.143	0.266	0.833	0.294
4	0.133	0.266	0.500	0.147
5	0.433	0.215	0.454	0.098
6	0.414	0.233	0.333	0.098
7	0.227	0.225	0.100	Trace
8	0.080	0.266	Trace	Trace
9	0.123	0.416	Trace	0
10	0.100	0.209	0	4.955
11	0.066	0.225	4.938	
12	0.100	0.200		
13	0.107	0.234		
14	0.147	0.294		
15	0.147	0.225		
16	0.075	0.171		
17	0.098	0.215		
18	0.113	0.215		
19	0.098	0.107		
20	0.110	0.178		
21	0.133	0.125		
22	0.215	0.125		
23	0.215	0.075		
24	0.215	Trace		
25	0.187	Trace		
26	0.107	0		
27	0.115	4.918		
28	0.147			
29	0.125			
30	0.125			
31	0.125			
32	0.090			
33	Trace			
34	Trace			
35	0			
	4.984			

This experiment indicates that of the methods tested the extraction with hot chloroform after alkalinizing with sodium bicarbonate is the most efficient, requiring only eight extractions of 10 c.c. each. As a result of all these preliminary tests the final method used for the analysis for morphine sulphate in nutrient agar was as follows:

The nutrient agar is broken up as well as possible into small pieces and is mixed with 2 per cent tartaric acid solution in proportion of 5 times

as much reagent as agar. The entire is well mixed and allowed to stand overnight after which the soluble tartrate is filtered off, the agar residue being washed about three or four times with distilled water. The combined filtrate and washings are evaporated over a water-bath down to 10 to 15 c.c. to which is added dry sodium bicarbonate slightly in excess of the point where effervescence ceases. To this is added 10 c.c. of pure chloroform, the mixture shaken and heated for 5 minutes over an electric hot plate. For this purpose a simple modified Wiley condenser is used to prevent evaporation and loss of the chloroform. After cooling, the chloroform layer is removed in a separatory funnel and the aqueous part is extracted for 7 or 8 times more. The total chloroform extracts are combined, diluted to a definite volume, and fractions thereof used for colorimetric comparison after evaporating off the chloroform and adding Marquis' reagent to the residue. A point in technic that has been important in obtaining satisfactory results is that no trace of acid be present in the glassware into which the chloroform extracts are placed. In order to avoid this error all the glass utensils were rinsed just before use with a small amount of weak ammonia water.

Colorimetric Analysis and Determinations

The colorimetric analysis and determinations were made as follows:

1. The standard of morphine sulphate is prepared from an aqueous solution containing 1 milligram per one cubic centimeter. This is placed in a small beaker and evaporated to dryness on the water-bath. A portion of the unknown in chloroform is placed in another beaker and likewise evaporated to dryness. When both residues, the known and unknown have cooled to room temperature, 10 cubic centimeters of a freshly prepared Marquis' reagent are added to each simultaneously and stirred until the color is uniformly diffused throughout the fluid. An assistant is employed to stir one of the beakers. If the color of the unknown is considerably deeper than the known, definite amounts of Marquis' reagent can be added until the colors more nearly match, or vice versa. The colors are compared in the colorimeter (in this work the Kober colorimeter proved entirely satisfactory) and the results are calculated with the following formula.

$$\frac{K}{X} \times S \times N \times \frac{V}{P} = \text{Mg. alkaloid in unknown as morphine sulphate.}$$

K = the reading of the standard tube, this may conveniently be placed at 10.0;

X = the reading of the tube containing the unknown;

S = the strength of the standard solution. If 1 mg. of morphine sulphate has been dissolved in 10 c.c. Marquis' reagent, then the standard solution is 0.1 mg. alkaloidal salt per c.c. of solution;

N = the number of c.c. of Marquis' reagent used to dissolve the unknown;

V = the total chloroform extract and,

P = the portion of the total chloroform extract used in the test.

Example.—The total combined chloroform extract is 100 c.c., of this 15 c.c. is evaporated to dryness and the residue dissolved in 20 c.c. Marquis' reagent. The standard solution contains 1 mg. of the alkaloid salt in 10 c.c. reagent. The

standard tube is set at 10 and the unknown reads 12.5. We have then V = 100; P = 15; N = 20; S = 10; K = 10; X = 12.5 or

$$\frac{10.0}{12.5} \times \frac{1}{10} \times \frac{20}{1} \times \frac{100}{15} = 10.666 \text{ mg. morphine sulphate in the unknown.}$$

By this method a series of nutrient agar tubes containing known amounts of morphine sulphate which was added to the agar have been analyzed with the results given in Table VI.

TABLE VI

ANALYSIS AND COLORIMETRIC ESTIMATION WITH MARQUIS' REAGENT OF MORPHINE SULPHATE IN NUTRIENT AGAR OF KNOWN ALKALOID CONTENT

AMOUNT OF MORPHINE SULPHATE IN MILLIGRAMS ADDED TO 20 GRAMS NUTRIENT AGAR MEDIUM	AMOUNT OF MORPHINE SULPHATE IN MILLIGRAMS RECOVERED BY ANALYSIS	
	AGAR MIXTURE No. I.	AGAR MIXTURE No. II.
32.0	27.2	31.5
16.0	15.5	15.3
8.0	7.6	7.8
4.0	3.5	3.7
2.0	1.97	1.92
1.0	0.94	0.96
0.50	0.42	0.44
0.10*	0.08	0.08

*A small amount of organic matter present in the final extract giving a brownish color with H_2SO_4 precludes the accurate determination of amounts below this.

The above described method of analysis for morphine sulphate in an organic colloidal mixture (nutrient agar medium) yields quantitative results down to amounts as small as 0.1 milligram in 20 grams of agar, below this amount the recovery is less perfect.

In order to test the accuracy of this method for the quantitative determination of morphine in tissues, beef muscle and pig liver to which had been added 5 milligrams of morphine sulphate were analyzed by the above described method which was slightly modified in that the tissues were mixed with 10 volumes of 3 per cent trichloracetic acid and boiled for 10 minutes instead of mixing them with 2 per cent tartaric acid. Trichloracetic acid

TABLE VII

THE QUANTITATIVE ANALYSIS OF TISSUES FOR MORPHINE BY THE COLORIMETRIC METHOD WITH MARQUIS' REAGENT

SAMPLE ANALYZED	RECOVERY OF MORPHINE SULPHATE FROM 5 GRAMS OF FRESH TISSUES TO WHICH WAS ADDED 5 MILLIGRAMS OF THE ALKALOID SALT	
	BEEF MUSCLE	PIG LIVER
No. 1	4.76 milligrams	4.58 milligrams
No. 2	4.77 milligrams	4.76 milligrams
No. 3	4.67 milligrams	4.88 milligrams

was chosen because it was found in preliminary experiments to be just as satisfactory as 2 per cent tartaric acid in so far as the extraction of morphine was concerned, and as was originally found by Greenwald[4] it was a far more perfect tissue coagulant or protein precipitant. The results of these tissue analyses are given in Table VII.

An examination of Table VII reveals that morphine sulphate added to fresh tissues (beef muscle and pig liver) in 5 milligram amounts and immediately analyzed can be recovered practically quantitatively by the colorimetric method using Marquis' reagent as described above.

SUMMARY

A quantitative colorimetric method for the estimation of morphine sulphate in tissues and organic colloidal mixtures is described.

There is a preliminary precipitation of the proteins by means of ten volumes of 3 per cent trichloracetic acid and subsequent extraction with hot chloroform.

The color utilized in this reaction is the purple red reaction with Marquis' reagent which is evanescent. The standard color is prepared by adding a known amount of the alkaloidal salt to a known volume of Marquis' reagent, similarly and simultaneously with the preparation of the unknown.

By means of this method we have been able to extract quantitatively morphine sulphate from tissues and colloidal solutions in amounts from 0.10 to 50.0 milligrams and to determine it colorimetrically in amounts as low as 0.003 milligrams.

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THE HECHT-GRADWOHL TEST EMPLOYING ICE CHEST FIXATION. A PRELIMINARY REPORT

By H. D. McIntyre, M.D., E. A. Worth, M.D., and A. P. McIntyre, A.B., Cincinnati, Ohio

WHEN the ice chest method of complement fixation began to be in vogue it occurred to us that this method might yield good results in the Hecht-Gradwohl test. We had seen that the Wassermann reaction employing complement fixation in the ice chest at 2° C. yielded a considerable number of positives over the water-bath Hecht-Gradwohl test even though acetone-insoluble antigen were used.[1] We then decided on a comparison of the relative merits of the Wassermann and Hecht-Gradwohl test with the complement fixation taking place in the ice chest in both.

The following technic was worked out:

TECHNIC OF THE HECHT-GRADWOHL TEST WITH COMPLEMENT FIXATION IN THE ICE CHEST AT 2° C.

Eighteen tubes are used in each test. Ten tubes are placed in the first row. These tubes are used to determine the hemolytic index as outlined by Gradwohl.[2] Four tubes are used in the second row, three containing plain antigen and the remaining tube being the control. Four tubes are used in the third row, three containing graded amounts of cholesterolized antigen and the other tube being the control.

Each of the eighteen tubes receives .1 c.c. of the patient's serum, not over twenty-four hours old. The best results are obtained with fresh serum. The first tube of the first row receives .9 c.c. of .9 per cent salt solution, the second tube .8 c.c., the third tube .7 c.c. and so on, the last tube in that row receiving no salt solution.

The second row receives plain antigen as follows; the first tube two units of antigen, the second tube one and one-half units, the third tube one unit, the fourth tube receives no antigen as it is the control. All tubes are brought to a volume of one c.c. with salt solution.

The third row receives cholesterolized antigen as follows; two units in the first tube, one and one-half units in the second tube, and one unit in the third tube. The fourth tube receives no antigen as it is the control. All tubes are then brought to a volume of one cubic centimeter with salt solution.

All tubes are now placed in the ice chest for a period of ten hours at 2° C. This allows the complement to be fixed in the antigen containing tubes in case the serum is a positive one. At the end of the ten hour period the ten tubes of the first row are removed from the ice chest and the hemolytic index of the serum is determined in the manner outlined by Gradwohl, that is, .1 c.c. of a 5 per cent suspension of washed sheep cells is added to the first tube, .2 c.c. to the second tube, .3 c.c. to the third tube and so on, the tenth tube receiving 1 c.c. of the suspension of cells. These tubes are then placed in the water-bath for thirty minutes at 37.5° C. The highest tube in the series showing complete hemolysis is taken as the index. Following the determination of the index the antigen containing tubes are removed from the ice chest and one-half of the amount of cells represented by the index is added to each tube in the second and third rows. The tubes are then placed in the water-bath for one-half hour at 37.5° C. If all tubes clear, the test is negative. If no hemolysis occurs in the antigen containing tubes, the control being clear, the test is positive.

One hundred sera were subjected to the foregoing technic together with the classical Wassermann with complement fixation in the water-bath and ice chest and the classical Hecht-Gradwohl with complement fixation in the water-bath. Fifty of these sera were from luetic patients, fifty were from nonluetic patients. The sera from the nonluetic patients reacted negatively to all tests. The comparative percentages of the positive reactions yielded by the different methods employed in the examination of the fifty luetic sera are given in Table I.

It will be seen that the Hecht-Gradwohl test employing complement fixation in the ice chest yields a higher percentage of positive reactions than do

TABLE I

	PERCENTAGE OF POSITIVE
1. The Wassermann test employing complement fixation in the water-bath for thirty minutes at 37.5° C. using plain antigen	23.07
1'. The Wassermann as in (1) except that cholesterolized antigen is used	34.20
2. The Hecht-Gradwohl test with complement fixation in the water-bath at 37.5° C. for thirty minutes using plain antigen	34.20
2'. The Hecht-Gradwohl test as in (2) except that cholesterolized antigen is used	53.08
3. The Wassermann test employing complement fixation in the ice chest at 2° C. for ten hours using plain antigen	73.00
3'. The Wassermann test as in (3) except that cholesterolized antigen is used	77.00
4. The Hecht-Gradwohl test employing complement fixation in the ice chest for a period of ten hours at 2° C. using plain antigen	80.70
4'. The Hecht-Gradwohl test as in (4) except that cholesterolized antigen is used	86.90

any of the other methods above tabulated. There are several reasons why this is so.

Complement in human serum is sometimes an unstable thermolabile substance which will deteriorate even if subjected to a temperature of 37.5° C. for a period of one-half hour. In the Hecht-Gradwohl test this is what happens if the complement fixation takes place in the water-bath. We have encountered ten sera in which such deterioration of complement has occurred. This was determined by placing a serum in the water-bath for one-half hour titrating a portion of it at the same time and titrating a second portion which had been subjected to the water-bath temperature. Ten sera yielded a lower index on the second titration than on the first. This source of error is obviated if complement fixation is carried out in the ice chest as the antigen containing tubes are then subjected to the water-bath temperature for only one-half hour.

Furthermore, this test has all of the added advantages pointed out by Gradwohl in addition to the one just alluded to, as well as the advantage of ice chest methods of complement fixation which we have emphasized in an earlier paper.[3] Theoretically the Hecht-Gradwohl test with ice chest complement fixation should be the test par excellence for the detection of antiluetic amboceptor in human serum. Practically, however, in a large series of cases, the ice chest Wassermann and the ice chest Hecht-Gradwohl tests would agree in nearly all instances.

A glance at our table will show that our percentage of positive reactions in the series of the fifty luetic patients is comparatively low. The reason is as follows: the fifty patients selected for the experiment represented cases of latent lues and lues under treatment where we had reason to know from previous serologic examination that their serums contained only small amounts of the antiluetic reacting substance. Such a series serves well to show the comparative value of serologic reactions, whereas if secondary untreated cases had been used, all the various methods would have yielded positive reactions.

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THE PRECIPITIN REACTION USED AS EVIDENCE FOR THE IDENTIFICATION OF HUMAN BLOOD IN AN AMERICAN COURT

By John B. Ekeley, Ph.D., Sc.D., Boulder, Colorado

THE writer is not aware of any recorded case in which the results of the application of the precipitin reaction have been used in an American court of justice as evidence for the identification of human blood. Many cases are recorded of its use in Europe, especially in Germany. It therefore seems proper that the following case in the recent experience of the writer should be available for reference.

In September, 1914, J. T., a rancher living near Raton, New Mexico, was charged* with murdering his wife. The evidence against him was all circumstantial, a part of which was a "jumper" and overalls which he had worn on the day of the crime, and from which it was charged attempts had been made to remove blood spots by cleaning, and a carefully cleaned iron poker which the State charged had been used in committing the crime.

The writer was asked to determine whether or not the spots on the clothing were due to the presence of human blood, and also to examine the poker for any traces thereof.

Examination of the clothing showed thirty-four suspected spots, among which were several minute dark red, shiny globules adhering to roughened portions of the lower part of the leg of the overalls. The spots were carefully cut from the fabric, the excess of cloth cut away, and carefully shredded to pieces by means of a needle. The poker was made of a piece of iron rod one end of which had been bent and twisted around part of the remainder so as to form the handle. It had the appearance of having been carefully cleaned. Examination with a magnifying glass, however, showed in one of the depressions between the twisted metal a small amount of dark red, shiny material which had the appearance of dried blood. This was picked out carefully with a needle. It weighed .019 grams.

Examination of the Spots.—The finely shredded cloth containing the spots from the clothing was placed in a sterilized test tube together with 2 c.c. physiologic salt solution containing a trace of phenol. After remaining in an incubator at body temperature for 48 hours, the liquid was filtered off through a hard filter by suction, giving a water clear filtrate of neutral reaction. This was divided into two equal portions. To one portion was added 1 c.c. of rabbit's serum† immunized to human blood and sensitive to dilutions of 1:3000. The reaction was characteristic, the cloudiness forming within a few moments, with the subsequent coagulation and settling to the bottom of the tube. The immunized serum gave no precipitate with the physiologic salt solution alone, with diluted rabbit's blood, with guinea pig's

*The case was bitterly fought, dragging through six years, coming to trial twice, the first trial resulting in conviction, the second, granted by the Supreme Court after appeal, resulting in acquittal.

†The immune serum used was obtained by Dr. R. C. Whitman of the University of Colorado Medical School from Prof. Ludwig Hektoen of Chicago.

blood, or with dog's blood. To the other portion of the filtrate was added serum from a rabbit which had not been immunized against human blood. No precipitate appeared.

The conclusion was that the spots contained human blood and testimony was so given. Counsel for the defense took care on cross examination to bring out the fact that the precipitate might have been caused by the presence in the spots of dried body secretions other than blood, naming specifically serum from the nose, and seminal fluid. The fact that the blood of an anthropoid ape would also give the reaction was also brought out, but on the whole the defense did not attempt to throw any doubt upon the reliability of the precipitin reaction.

Examination of the Material from the Poker.—The material scraped from the poker was similarly extracted with 1 c.c. physiologic salt solution at body temperature and filtered to a water clear filtrate. This was also neutral in reaction. Addition of .1 c.c. of the immunized serum gave a characteristic precipitate as before, the conclusion being that the material from the poker contained human blood and testimony was so given.

MERCURY MANOMETER FLOAT THAT WILL RIDE ON THE SURFACE OF THE MERCURY THE SAME AT ALL TIMES*

By M. A. Blankenhorn, M.D., and E. J. Warnick, Cleveland, Ohio.

THIS mercury manometer float is made of hard rubber and has three very good features:

1. The conical shape, which on each upward stroke will rid itself of the mercury which happens to get above the float.

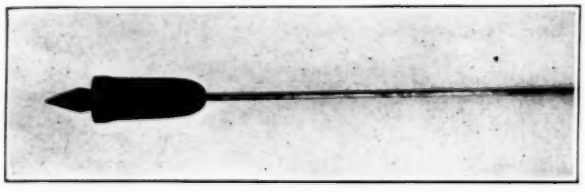

Fig. 1.

2. The flat base, which rides on the surface of the mercury.

3. The projection on the bottom, with a very small neck, which makes the mercury form a collar around it, thereby holding the float.

*From the Medical Research Laboratory, Lakeside Hospital, Cleveland, Ohio.

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EDITORIALS

Notes on the Vitamins

PLIMMER[1] observed four young pigs presenting the following symptoms: (1) Arrest of growth. (2) Evidence of pain leading to frenzy when touched. (3) Marked lassitude and absence of appetite. (4) Spasmodic twitching of limb muscles. (5) Swollen joints. (6) Failure to coordinate movements and dragging of the limbs in some cases.

On inquiry it was ascertained that these animals were being fed on a mash composed of turnips, meal and grits, all thoroughly cooked. Cooking of the food was discontinued and the same mash, with the addition of a larger proportion of turnip fed in the raw state. The turnips were fed directly after being plucked from the fields. Occasionally some skimmed milk or buttermilk was added to this food. After a few meals of raw food the appetite showed improvement and after fourteen days the pig least affected seemed quite normal. The others recovered slowly, but within from six to eight weeks, all were accumulating fat and growing normally. Plimmer states that this disease is fairly common among pigs in England, that it is frequently referred to as "rheumatism," sometimes as "rickets" or "pig gout," and that it has been found that raw potatoes generally cure it. In due time these

animals were sold at current prices and were slaughtered. The bones of one came under Plimmer's observation and he found that, while the ribs on one side appeared normal, on the other, one showed a healed fracture and five showed hemorrhage; two of these were curved. The leg bones on one side were normal, while those on the other showed thickening of the radius and ulnar and fusion and thickening of the tibia and fibula.

Hart, Steenbock and Ellis[2] have published an investigation of the antiscorbutic potency of milk powders. There are three processes commercially operative in the preparation of milk powder: (a) The spray process as employed by Merrell and Soule; (b) the spray process in which the powder is removed and cooled a few seconds after being dried, employed by the California Central Creameries; (c) the drying of milk on heated rolls, known as the Just process. In testing the antiscorbutic potency of these milk powders, several things must be taken into consideration. In the first place, if the milk be poor on account of inadequate and improper food supplied to the cow it is evident that converting it into a powder will not increase its vitamine content. In the second place, under natural conditions the vitamine content of summer milk, especially when the cows feed upon green pastures, is greater than that of winter milk. It is reasonable, therefore, to conclude that any results obtained in experiments of this kind cannot be considered as having standard value. The authors summarize as follows: "(1) Milk powders vary in their antiscorbutic properties. Aside from the factor of the initial quantity of this vitamine in the milk as influenced by feeding, the powders vary in their potency with the process used in their manufacture, the spray process of manufacture being more destructive of the antiscorbutic vitamine than the Just process. (2) These results should in no way condemn the milk powders made by spray processes. They only point out their limitations when used as the sole source of nutrients in infant feeding. (3) Probably with all milk powders, irrespective of method of manufacture, the safest procedure in a restricted dietary, particularly in infant feeding, is to supplement them with some potent source of the antiscorbutic vitamine. A possible exception to this statement would apply to the powders made by the Just process from summer-produced milks or even from winter-produced milks where the cow's ration is made rich in antiscorbutic vitamine by the proper selection of roots and tubers."

We are inclined to be a little more cautious than the statements made under (3) as above quoted. When the child is fed upon milk powder, whatever the process employed in its manufacture or the season of the year when it is produced, the presence of sufficient of the antiscorbutic vitamine should be made certain by the administration of orange juice.

The same authors report their observations on the stability of the antiscorbutic vitamine and its behavior to various treatments. Numerous investigators have sought for methods of drying vegetables without reduction in their antiscorbutic properties. This investigation is especially concerned with cabbage. It was thought that possibly by drying cabbage in an atmosphere of carbon dioxid at as low a temperature as possible, its antiscorbutic vitamine might escape injury. The desiccation was carried out in such an

atmosphere at 65° C., but it was found that this does not prevent the destruction of the antiscorbutic substance. A second purpose was to study the influence of fermentation as in the manufacture of sauerkraut. Again, it was found that this process leads to the destruction of the antiscorbutic factor. Incidentally, it was ascertained that the antiscorbutic factor is destroyed, or at least greatly reduced, in the preparation of silage. This last finding is probably of more importance than that regarding sauerkraut. If the vitamine in silo is destroyed, this food becomes less valuable not only to the cattle eating it, but it affects and lowers the value of the milk as a food for children.

Drummond[3] comments upon the confusion which has arisen on account of the different terms employed in referring to the essential food factors which have no caloric value. In his classical paper published in 1912, Hopkins designated these bodies as "accessory factors of the diet." A little later, Funk, in his experimental studies on beriberi chose the word "vitamine." The criticism usually raised against Funk's word is that the termination "ine" is one employed in chemical nomenclature to denote substances basic in character. In order to avoid this objection it has been suggested that the word "vitamine" be written without the final "e" and this has been approved by the British Chemical Society. McCollum introduced the terms fat-soluble A and water-soluble B. This has added to the confusion. Drummond now proposes that we use vitamin A, B, C, etc. He thinks that this simplified scheme will be sufficient until these bodies are isolated and their true nature determined.

Drummond and Coward[4] state that much of the confusion and apparent disagreement found among writers on vitamins is due to failure to have a properly purified basal diet absolutely devoid of vitamins. They state that commercial caseinogen contains relatively large amounts of the fat-soluble vitamin and should never be used as a part of the basic diet until it has been carefully purified. They heat their commercial caseinogen for twenty-four hours or more in shallow dishes at a temperature of 102° C., after which they subject it to prolonged extraction with both alcohol and ether. Purified rice starch is almost entirely devoid of fat-soluble A and is suitable for the basic diet without complicating and costly preliminary extraction. For the fatty constituent these investigators employ a fully hardened and refined vegetable oil, usually cotton seed oil. This consists largely of tristearin and is entirely devoid of vitamin A. Orange juice and salt mixture, as well as yeast extract, are devoid of vitamin A. The composition of the basic ration employed by Drummond and Coward is as follows:

Purified caseinogen	18	parts
Purified rice starch	52	"
Refined vegetable oil	15	"
Yeast extract	5	"
Orange juice	5	"
Salt mixture	5	"

Rats fed upon this diet differed in behavior somewhat according to their age. For experiments on growth, rats from four to five weeks old and weighing from 50 to 70 grams should be selected. On the above given diet such animals show but little growth and soon cease to grow. Any considerable increase of body

weight in these animals is interpreted as an indication that the basic ration is not sufficiently purified. Older and larger rats are unsatisfactory in testing for vitamin A and its effects.

With the above basic diet, rats weighing from 50 to 70 grams were fed, with the addition of certain nuts, namely, almonds, butter nuts, walnuts, peanuts, Brazil and Barcelona nuts. The results showed that these nuts have only small amounts of vitamin A associated with their fats. This is confirmation of the conclusion reached by McCollum and others, that vitamin A is formed largely in the green part of living plants and is not stored to any appreciable extent in seeds or other resting tissues.

These investigators, on the same basic diet, have tested for vitamin A, both in vegetable and animal fats, and state their conclusions as follows: "(1) No hard and fast line can be drawn between the animal and the vegetable oils and fats when their value as a source of vitamin A is being considered. (2) Taken as a class the animal fats possess growth-promoting power superior to that of the vegetable oils, but we have observed that one or two members of the latter class (e. g. palm oil) may show considerable activity in that respect. (3) Unless we assume the existence of a leucoform it does not appear probable that the fat-soluble vitamin is a member of the lipochrome class of pigments. The frequent association of the growth factor with pigments of that type must therefore be regarded as accidental. (4) The nutritive value of an animal oil or fat would appear to be influenced considerably by the diet of the animal. One preliminary experiment shows that the winter feeding of cows may have the effect of lowering the food value of the milk unless considerable care is exercised in the selection of the animal's diet."

Zilva[5] has apparently demonstrated the following: Alcohol extracts from carrots and cabbage the fat-soluble factor A; the alcoholic extract for from ten to twelve grams of fresh carrots given daily is sufficient to promote normal growth in rats subsisting on a diet wholly wanting in the fat-soluble factor; the alcoholic extracts from carrots contain the antineuritic and to a smaller extent the antiscorbutic factors; an ethereal extract from the alcoholic fraction equivalent to twenty-five grams of fresh carrots promotes recovery and renews growth in rats declining in weight on account of a fat-soluble deficiency.

Stephenson and Clark[6] have made a special study of the eye involvement which appears in some rats fed upon a diet deficient in vitamin A. This eye complication was first observed by McCollum and Simmonds in 1917 and was designated xerophthalmia, but it is now most frequently described under the name keratomalacia. It is worthy of mention that a similar disease was reported by Mori in Japan in 1904 among children who were suffering from fat starvation. Mori found that it was cured by feeding on chicken livers and fish oils. He made the further observation that it occurred among populations using vegetable oils but was never seen among people using fish oils. A similar disease was reported in Copenhagen by Bloch in 1917 among children fed upon highly skimmed milk. Bloch found that these children were cured by feeding upon whole milk and cod-liver oil. Stephenson and Clark find that keratomalacia occurred in twenty-eight per cent of their rats kept on a diet

free from vitamin A and that the eye complication rapidly disappeared when the deficiency was made good. They state their findings as follows: "(1) Corneal disease is not coincident with failure of growth culminating in death; it forms only twenty-eight per cent of the cases examined. (2) This disease is further differentiated from the cessation of growth-death symptoms by occurring at a later stage in the experimental period and rising more rapidly to a maximum. (3) At no period in the experiment are all the deaths preceded by eye disease or all the survivors afflicted with it. (4) Cure of the corneal disease was affected by the replacement of the fat-soluble factor in one hundred per cent of the cases attempted." They failed to find any specific bacterium associated with the local disease in the eye. Histologically, the first evidence of the eye being affected is the presence of numerous leucocytes in the cornea. This leucocytic infiltration is accompanied by or soon followed by the presence of bacteria which are diverse in kind. It appears, therefore, that the absence of vitamin A lowers the vitality of the corneal tissue and while this condition is in operation any bacteria that may be present begin operation. The cure of the eye complication is in no way hastened or modified by local application, but disappears wholly within a few days after return to normal diet. It will be understood that the keratomalacia may go so far as to lead to perforation of the cornea and destruction of the eyesight. Of course, return to proper food after this stage has been reached will not restore the sight, but does arrest the inflammatory processes. This appears to be a striking instance of the relation between lowered nutrition and bacterial infection. In this case the nutrition of a specific tissue is so reduced by lack of an essential food factor that any bacterium which finds access to the tissue in this condition takes on pathogenic qualities.

Stephenson[7] has studied the relation between certain yellow plant pigments and vitamin A. In 1914 it was shown by Palmer and Eckles that the coloring matter in milk and in the body fats of cows consists essentially of carotin with only traces of xanthophyll. The same substance is found to be the chief coloring matter in the fats of certain other herbivorous animals, especially the horse. Strange to say, however, carotin cannot be found in the blood serum or fats of swine and only traces in the corresponding tissues of sheep and goats. The pigments stored by birds and found in the yolk of the egg and in the body fats is principally xanthophyll. Quite naturally, one comes to regard a yellow milk as a rich milk and is inclined to judge of the food value of the milk by its color. The researches of Stephenson seem to show quite conclusively that vitamin A is not carotin and that the color of milk is not an indication of its richness in vitamin A; in fact, the coloring matter of butter fat may be completely removed or destroyed by filtration through charcoal without in the least affecting the vitamin content of the butter.

Hopkins[8] has shown that vitamin A in butter is highly resistant to heat, provided aeration is excluded. Butter may be heated to 120° C. in the absence of air for four hours and even longer without any marked reduction in its vitamin content, as demonstrated by rat feeding provided the fat forms fifteen per cent of the food; on the other hand, aeration for four hours at 120° destroys the greater part of the vitamin in butter. It will be understood that

aeration at high temperatures leads to more speedy destruction of the vitamin than aeration at low temperature, but even at ordinary room temperature butter exposed in thin layers loses much of its vitamin A within a few days. The housewife has learned by experience that butter keeps better in large masses at low temperatures and with the exclusion of light. It seems that we have unconsciously through generations learned many things as to the preparation and preservation of food which only recently science has demonstrated.

Since vitamin A is destroyed by aeration, it is plain that exposure of this food to an ozonized atmosphere is detrimental. This has been scientifically demonstrated by Zilva, who has shown that when butter is spread on a thin layer and exposed to ultraviolet rays the fat-soluble factor is inactivated.

There has been some discussion among experts as to the presence of vitamin A in lard. Numerous experiments have been made and generally the conclusion has been reached that the body fat of the hog is devoid of this essential food factor. Drummond and his assistants have gone through this subject again and they have found that the amount of vitamin A in lard depends to a considerable extent at least, upon the food upon which the hog has been reared and the process employed in rendering the lard. They conclude their work with the following statements: (1) "The pig is able to store up supplies of vitamin A in the body fat when fed upon a diet containing ample supplies of that factor, as for example when grass is fed. (2) When the diet of the pig is deficient in vitamin A, as for example when the diet consists almost entirely of toppings and whey, no appreciable amounts of that dietary factor can be detected in the body fat. (3) The processes employed in the manufacture of lard on a large scale cause a very marked destruction of the vitamin present in pig fat."

There have been many attempts to connect deficiency diseases with disorders of some endocrine gland. Nothing very substantial has come from these suggestions. Now, Cramer comes along with the suggestion that certain fat formations in the body are really glandular tissue and that these are concerned in some way with the functioning of the vitamins. He says: "In all types of mammals which have been investigated there exists a glandular type of adipose tissue histogenetically distinct from the ordinary adipose. It has been described under various names, e. g., 'primitive fat organ,' 'fat gland,' hibernating gland,' 'brown fat,' 'interscapular gland.' In all species it has in the embryo a characteristic gland-like structure. In some species (white rat, tame mouse, hibernating animals) it retains this characteristic histological structure. But in most species the tissue acquires the appearance of ordinary adipose tissue soon after birth, so that the process has been looked upon as one of the stages leading to the formation of the adipose tissue of the adult organism. It is shown that this tissue, which is very vascular, is functionally distinct from ordinary adipose tissue. The fatty material which it contains is rich in cholesterin compounds and other lipoids in addition to ordinary true fat. This load of lipoid is retained under conditions which bring about the disappearance of the ordinary adipose tissue. Evidence is given to show that it is functionally related to the thyroid and adrenal glands. A particularly close relationship exists between the lipoids of this tissue and those of the adrenal

cortex. The significance of the changes of the lipoids in the adrenal cortex is discussed. If vitamins are completely withheld from the diet the lipoids disappear from the adrenal cortex and from this tissue, which persists, however, as a tissue and exhibits the appearance of a very vascular endocrine organ. The existence of this tissue throws fresh light on the problem of deficiency diseases, since disturbance of the functional activity of this gland-like tissue will have to be considered as a factor in the etiology of these diseases. In view of its appearance its function and its importance it is proposed to call this glandular type of adipose tissue the 'lipoid gland' or 'cholesterin gland.' "

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—*V. C. V.*

Superinfection in Syphilis in Its Relation to Subtreatment

IT is generally held that a person infected with the virus of syphilis becomes practically immune to a second infection; that, with the development of the initial lesion, a condition becomes established which makes it difficult or impossible to superimpose a second infection upon the one already present and that this refractory state is maintained as long as an infection exists. Both human and animal experiences agree upon this, so far as they have been tested. In the absence of any evidence to the contrary it has been assumed that the principles contained in this conception of syphilitic immunity apply to treated as well as to untreated cases of syphilis and many syphilographers have regarded the appearance of fresh lesions of the chancre type, under circumstances which would indicate a new infection, as the most conclusive evidence of the cure of the previous infection. But Jacobi suggested that the reaction to a second infection may be viewed more as the expression of any existing state of immunity than as evidence of the presence or absence of infection.

There are matters that need elucidation and the need is accentuated by the fact, so stated by Brown and Pearce, that until the introduction of modern methods of spirocheticidal therapy, instances of so-called reinfection were comparatively rare, and interest in the subject was largely a theoretic affair. The point at issue, say Brown and Pearce, is not so much a question of the immunity conferred by syphilitic infection as it is the effect which certain therapeutics may have upon the resistance of infected individuals, and upon any spirochetes which may survive their action. This is the problem which Brown and Pearce have approached experimentally, for the purpose of dis-

covering the effect of subcurative doses of arsphenamine and neo-arsphenamine upon the resistance of infected animals to reinoculation.

These experiments consisted of the infection and treatment of two sets of rabbits—one with arsphenamine; the other with neoarsphenamine—after which they were reinoculated with luetic virus for the purpose of determining their susceptibility to a new infection. The experiments were rigorously controlled. Animals after being inoculated with the syphilitic virus and the lesions had developed were given subcurative injections of the two drugs, after which they were subjected to reinfection.

The effect of the treatment used in the experiments was as follows; Following the administration of the drugs the lesions began to regress, resolution proceeding somewhat more rapidly in the animals treated with neoarsphenamine than in those treated with arsphenamine. The first evidences of relapse were noted between 14 and 17 days after treatment. Clinical relapse among animals treated with neoarsphenamine was more delayed than among those treated with arsphenamine. In all the animals of both series, however, relapse occurred, which proves that the infection was active, and that the treatment used subcurative.

For the effects of reinoculation of infected (but untreated) controls, animals with the most advanced lesions were selected, while those showing least progress were used as controls. In but two instances out of ten inoculations did any lesion result which gave evidence of being due to syphilitic infection.

Reinoculation of treated animals, on the other hand gave different results, i. e., all but two developed perfectly typical chancres associated with lymphadenitis. It is of interest to note that the relapses of the original lesions occurred in eight of the ten reinoculations at about the time when the superinfection lesions became well established.

The results of the treatment and reinoculation as a whole are stated as follows: Of the animals treated with arsphenamine and then reinoculated, the original lesions were completely resolved in but one instance, and relapses occurred within 33 days in four of the five animals. By reinoculation characteristic chancres were produced in all the animals of the group. The results with neoarsphenamine were not so uniform. In two animals the lesions were quickly resolved. Clinical relapses occurred in all but one animal in the group at from 14 to 24 days after treatment. Characteristic chancres were produced by a second inoculation in three of the five animals.

The results of the whole experiment stated in summary are: (1) that the treatment employed was insufficient to cure any of the therapeutic controls; (2) that the infected (and untreated) controls were highly refractory to a second inoculation; (3) that the treated animals were highly susceptible to a second inoculation and that though not cured of their original infection, reacted to the second inoculation with the formation of lesions indistinguishable from primary sores; and (4) that in certain instances the treatment given had rendered infected animals more susceptible to infection than the normal controls.

One wonders after reading these results, what effect on reinoculability mercury has, whether given alone or in conjunction with arsenic. This is suggested by the statement that evidences of reinfection were less frequent before the advent of arsphenamine therapy. One also wonders what influence upon the present generation of syphilitics the too-general undertreatment is having; and whether in the campaign for education of the laity some emphasis should not be placed on the fact that undertreatment of syphilis is perhaps as serious an affair as none, and that a few doses of arsphenamine gives little but a false sense of security.

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—*P. G. W.*

Goiter in Northern Michigan

IT HAS long been known that goiter is unduly prevalent in certain areas roundabout the Great Lakes, both in the United States and in Canada. Marine has told us that at one time the sheep-raising industry in Michigan was threatened on account of the prevalence of this disease among these animals but that among sheep, goiter was exterminated by the substitution of native for imported salt, the former containing enough iodin to prevent the development of goiter in the animals. Before we entered the war, Marine and Kimball made a survey of goiter at Akron, Ohio, and induced the authorities to provide for the administration of an iodid to all school children. We suppose that this experiment was interrupted by the war; at least, we have had no information concerning the result.

Levin[1] has reported upon the examination of 1,783 unselected persons in the Townships of Torch Lake and Schoolcraft in Houghton County, Michigan. One part of this community is supplied with spring water, a second part with Lake Superior water, and a third, with well water. However, there is no evidence that these waters differ materially either in organic or inorganic content. Levin states that in all these waters the relation of calcium to sodium elements is always more than 1:1, and that this relationship occurs but rarely in any other water-supply in the United States. Levin's observations throw no light upon the influence of water in the causation of goiter, because, while there may be quantitative, there are apparently no qualitative, differences in the waters used. The people are Americans, the larger percentage of French-Canadian extraction, and they are above the average in intelligence and in living conditions. There are no marked unhygienic surroundings. Each home has plenty of sunlight, good ventilation, and a lot of fair size for gardening. In the 1,783 persons, ranging in age from newborn to sixty-one years, 1,146 had enlarged thyroids, with 682 simple goiters, 420 adenomas, and 44 colloids. Among the people examined there are only two cretins, and the mother of each

[1]One Thousand One Hundred Forty-Six Goiters in One Thousand Seven Hundred Eighty Three Persons: Arch. Int. Med., 1921, xxvii, 421.

of these has a goiter. There are a few families with several morons, although Levin apparently has not made any scientific mental tests. Out of the total number examined, mothers alone had goiters in 802 instances; fathers alone in 12, and both parents in 183. In regard to the possibility of goiter being due to infection, the author makes the following statement: "In analyzing those who have focal infection and develop enlargements of the thyroid, whether becoming toxic or not, I am not free to believe that infection alone, or the infective agent, causes goiter. The presence of infected teeth or infected hypertrophied tonsils, or contagious diseases, or other infections will cause in thyroid patients, thyroiditis, enlargements of adenomas, or toxin symptoms, occasionally. The infective agent very rarely does this directly, but the focal toxemia causes a secondary effect on the thyroid. The weight of evidence and observations leads me to conclude that except for a direct thyroiditis, which is not uncommon, thyroid enlargements or thyroid zones are not caused necessarily by direct infective agents. This is said with due respect to the modern writers on the subject who lean toward some infective agent in itself being responsible for thyroid zones. The future will settle the question whether direct infection, or intestinal toxemia, or just the lack of some element in the soil or water, or uncleanliness in the handling of vegetables grown here is, or contains, the causative agent. The large numbers in our section who are practically normal and healthy otherwise, those who lose their enlargements of the thyroid during absence from this section, and the presence of enlargements which become more marked here than elsewhere during the periods of life when the greatest metabolic changes occur—as puberty, menopause, menstruation and pregnancy—are points in circumstantial evidence that offset much in the infective theory." We suggest to Levin that it would be a good idea to try in his community the administration of small doses of sodium iodid, as was proposed by Marine for the school children of Akron.

—*V. C. V.*

Editor Goes to National Research Council.

Attention is called to the change in address of the Editor-in-chief from Ann Arbor, Michigan, to National Research Council, 1701 Massachusetts Avenue, Washington, D. C. All communications concerning the reading matter of the journal should be sent to Dr. V. C. Vaughan at the above-given address after September 10, 1921.

INDEX TO VOLUME VI

AUTHORS INDEX

In this index following the author's name the full title of the subject is given as it appears in the Journal. Editorials are also included in the list and are indicated by (*E*).

A

ABLESON, MARJORIE. (*See* Culpepper and Ableson), 276, 415

ARNOLD, LOYD. Classification of streptococcus, 312

ATKINSON, H. V. A composite reagent for the determination of sodium chloride in urine, 160

B

BAILEY, CAMERON, V. Apparatus used in the estimation of basal metabolism, 657

BAUMBERGER, J. P. A simple shaking device, 222

BENEDICT, STANLEY, R. (*See* Franke and Benedict), 618

—. (*See* Theis and Benedict), 680

BERG, W. M. Determination of coagulable protein in serum, 223

BLANCHARD, EMILY. (*See* Ferry and Blanchard), 23

BLANKENHORN, M. A., AND WARNICK, E. J. Mercury manometer float that will ride on the surface of the mercury the same at all times, 710

BOYER, EDWARD, E. H. Benign tumors of the gastrointestinal tract, 339

BRERETON, MAE. (*See* Greeley and Brereton), 349

BRODERS, A. C., AND MAHLE, A. E. Primary lymphosarcoma of the stomach. A report of twelve cases, 249

BROOKS, HARLOW. An accurate method for the clinical determination of early arterial disease, 597

BROSIUS, W. L. (*See* Owen, Martin and Brosius), 47

BULGER, HAROLD, A. Blood changes in a case of hemophilia after transfusion, 102.

C

CHESNEY, ADAM M., AND SNOW, FRANK W. A report of an epidemic of influenza in an army post of the American Expeditionary Forces in France, 78

COOPER, A. R., AND GROOT, J. T. The exposure of the ciliary ganglion in the dog for use in experimental work, 639

COVEY, GEO. W. A review of ninety-four necropsies. With special reference to the pneumonias, 611

CULPEPPER, WILLIAM L., AND ABLESON, MARJORIE. Chaulmoogra oil in the treatment of tuberculosis, 415

—AND—. Report on five thousand bloods typed using Moss's grouping, 276

D

DUKE, W. W. Ice water-bath in complement fixation for the Wassermann reaction—a shortened technic, 392

E

EDDY, NATHAN B. A simple device for the demonstration of heart block in the student laboratory, 635

EGGSTEIN, A. A. The alkali reserve of blood plasma during acute anaphylactic shock, 555

—. The alkali reserve of the blood plasma during protein shock, 481

EKELEY, JOHN B. The precipitin reaction used as evidence for the identification of human blood in an American court, 709

ELLIS, ALLER G. An analysis of one hundred postmortem examinations in Siam, 199

F

FEINBERG, S. M. The value of the Ross-Jones test on bloody spinal fluid, 642

FERRY N. S., AND BLANCHARD, EMILY. Preparation and standardization of polyvalent antipneumococcic serum, 23

FRANKE, ELIZABETH, AND BENEDICT, STANLEY R. A method for the determination of blood volume, 618

G

GAUSS, HARRY. A colorimetric method for the estimation of morphine in colloidal mixtures and tissues, 699

GAY, FREDERICK P. Present day immunology, (*E*), 229

GOECKEL, HENRY J. The diagnosis of typhoid and paratyphoid infections, 335
—. The routine determination of cretinine and acetone in urine, 338
GOLDMAN, ALFRED. (*See* Mudd, Grant and Goldman), 175, 253, 322
GRADWOHL, R. B. H. The training and proper recognition of the laboratory technician, 644
GRANT, GARNET, B., AND WILSON, ERIC R. Two stains used in preference to Wright's stain in the routine staining of blood smears, 593
GRANT, SAMUEL B. (*See* Mudd, Grant and Goldman), 175 253, 322
GREELEY, HORACE, AND BRERETON, MAE. The bacteriology of chronic nontuberculous lung disease, 349
GREGG, ROTH. (*See* Owen and Gregg), 220
GROOT, J. T. (*See* Cooper and Groot), 639

H

HADJOPOULOS, L. G. A standard method for preparing and standardizing lipoidal antigens for the Wassermann test, 624
HAMILTON, HERBERT C. Hemostatic agents, 398
HANZLIK, P. J. Hemostatic agents and the spontaneous changes in coagulation time following hemorrhage, 59
HASKINS, HOWARD D., AND OSGOOD, EDWIN E. Modifications of Van Slyke's titration method for estimating the alkali reserve of blood, 37
HIGGINS, JOHN A. (*See* Nielson and Higgins), 388

J

JACKSON, DENNIS E. Isolation of the specific hormone of the posterior portion of the pituitary gland (*E*), 48
—. (*See* Mills, Raap and Jackson), 374
—. AND RAAP, G. An experimental investigation of certain features of the pharmacological action of salvarsan, 1
JONES, LLOYD R. A comparison of three methods of examining sputa for B. tuberculosis, 41

K

KAHN, R. L. A simple method for the removal of natural amboceptor from human sera, 218
—. Complement vs. amboceptor titrations in the Wassermann test, 153
—. The Wassermann test and its interpretation, 579
KATZ, LOUIS N. Factors modifying the duration of ventricular systole, 291
KIELEY, CHARLES E. James theory of the emotions in relation to the adrenal glands, 193
KILDUFFE, ROBERT A. "Variations in the Wassermann reaction"—A reply, 98
KLIX, H. C. A simple method of isolating bacteria from pathologic material, 104
KOLMER, JOHN A. A system of laboratory examinations and records, 505
KREMERS, E. D. Some personal experiences with epidemic respiratory diseases in the army, with some remarks on metrods of control, 25

L

LAMB, FREDERICK H. Apparatus for staining and drying slides, 101
LEVISON, LOUIS A. Unsuccessful result following transfusion with immunized blood in a case of infectious endocarditis, 191
LEWIS, NOLAN D. C. The pathology of influenza as seen in those with chronic mental disease, 531

M

MACCARTY, WM. CARPENTER. The relation of pathologists to the institutional practice of medicine, 331
—, AND MAHLE ARTHUR E. Relation of differentiation and lymphocytic infiltration to post-operative longevity in gastric carcinoma, 473
MACLEOD, J. J. R. Present-day methods for studying the problem of ventilation (*E*), 521
MAGATH, THOMAS BYRD. A test for early renal insufficiency, 463
MAHLE, A. E. (*See* Broders and Mahle), 249
—. (*See* MacCarty and Mahle), 473
MANN, F. C. A case of spontaneous acute and subacute peptic ulcer and carcinoma of the thyroid in a dog, 213
MARTIN, F. A. (*See* Owen, Martin and Brosius), 47
MASON, E. C. A preliminary report on blood coagulation, 195
—. The pharmacological action of lead in organic combination, 427
—, AND PIECK, CARL E. A pharmacological study of benzyl benzoate, 62
MASUCCI, PETER. A note on the effect of amino acids on the growth of tubercle bacilli, 96
MCGUIGAN, HUGH. The utilization of the ciliary ganglion for class work in the pharmacology of the eye, 161
MCINTYRE, AURELIA P. (*See* McIntyre, North and McIntyre), 233
—. (*See* McIntyre, Worth and McIntyre), 706

McINTYRE, H. D., WORTH, E. A., AND McINTYRE, A. P. The Hecht-Gradwohl test employing ice chest fixation. A preliminary report, 706.
MILLS, C. A. RAAP, GERARD, AND JACKSON, D. E. A note on the relation between the blood-coagulating and the smooth muscle-contracting properties of tissue extracts, 374
MORSE, WITHROW. (*See* Van der Heyde and Morse), 520
MUDD, STUART, GRANT, SAMUEL B., AND GOLDMAN, ALFRED. The etiology of acute inflammations of the nose, pharynx and tonsils, 175, 253, 322
MYERS, C. N. On the preparation of metal salts of thioglycolic acid, 359
MYERS, VICTOR C. Chemical changes in the blood in disease. VII Chlorides, 17
—. The hemoglobin content of the blood (*E*), 648

N

NEILSON, CHAS. HUGH, AND WHEELON, HOMER. Studies on the resistance of the red blood cells, 454, 487, 568
NIELSEN, CARL, AND HIGGINS, JOHN A. Observations on the pharmacology of some benzyl esters, 388
NORTH, EMERSON A. (*See* McIntyre, North and McIntyre), 233

O

OSGOOD, EDWIN E. (*See* Haskins and Osgood), 37
OWEN, R. G., MARTIN, F. A., AND BROSIUS, W. L. Bacterial vaccines—chloretone solution as a vehicle for their administration, 47
—, AND GREGG, ROTH. Lactose—determination of, in milk by colorimetric method, 220

P

PALMER, GEORGE T. Ventilation, weather, and the common cold, 602, 684
PIECK, CARL E. (*See* Mason and Pieck), 62
PRYER, R. W. The etiology of scarlet fever, 561

R

RAAP, G. (*See* Jackson and Raap), 1
—. (*See* Mills, Raap and Jackson), 374
RIEGER, JOHN B. The estimation of chlorides in whole blood, 44

S

SEELMAN, J. J. Observations on the quantitative nature of complement fixation, 144
SNOW, FRANK W. (*See* Chesney and Snow), 78

T

THOMAS, J. EARL. (*See* Wheelon and Thomas), 124
THEIS, RUTH C., AND BENEDICT, STANLEY R. Distribution of uric acid in the blood, 680

V

VAN DER HEYDE, H. C., AND MORSE, WITHROW. A modification of the technic of the vividiffusion method of Abel, 520
VAUGHAN, WARREN T. American relief work in Vienna (*E*), 51
—. Biochemical changes in traumatic shock (*E*), 405
—. Experimental influenza bacillus infection in man (*E*), 525
—. Influenza and tuberculosis (*E*), 105
—. Late sequelæ of encephalitis lethargica (*E*), 288
—. Negative Wassermann reactions in syphilis (*E*), 653
—. Roentgen ray therapy in hyperthyroidism (*E*), 284
VAUGHAN, VICTOR C. Are there two diseases included under the present diagnosis of smallpox? (*E*), 54
—. Are we in danger of typhus fever? (*E*), 347
—. Diseases of animals communicable to man (*E*), 594
—. Epidemiology of tuberculosis (*E*), 231
—. Goiter in Northern Michigan (*E*), 719
—. Influenza among the Lapps (*E*), 57
—. International organization and public health (*E*), 529
—. Mexican smallpox (*E*), 289
—. Notes on the vitamins (*E*), 711
—. Scurvy in Northern Russia (*E*), 168
—. The control of measles (*E*), 114
—. The dietetic treatment of diabetes mellitus (*E*), 57
—. The effects of deficient dietaries on monkeys (*E*), 170
—. The life-history of the first case of myxedema treated by thyroid extract (*E*), 55
—. The spread of bacterial infection (*E*), 471
—. Two recent papers on pellagra (*E*), 654

W

WARNICK, E. J. (*See* Blankenhorn and Warnick), 710
WATSON, THOMAS. (*See* White and Watson), 45
WEBB, GERALD B. Influenza and tuberculosis—a postscript (*E*), 651
—. The route of absorption of inhaled substances (*E*), 341
—. Tuberculosis and reinfection (*E*), 162
—, AND C. T. R. What we know and do not know about tuberculosis (*E*), 112

WHEELON, HOMER, AND THOMAS, J. EARL. Observations on the motility of the antrum and the relation of rhythmic activity of the pyloric sphincter to that of the antrum, 124

—. (*See* Neilson and Wheelon), 454, 487, 568

WHITE, H. L., AND WATSON, THOMAS. A note on the stability of drawn blood, 45

WILSON, ERIC R. (*See* Grant and Wilson), 593

WOOLLEY, PAUL G. Blood sugar tolerance in cancer and in hypertension (*E*), 227

—. Complications of the arsphenamine treatment of syphilis (*E*), 344

—. Roentgenology and the internist (*E*), 53

—. Silver arsphenamine (*E*), 527

—. Superinfection in syphilis in its relation to subtreatment (*E*), 717

—. Syphilology and clinical synthesis (*E*), 469

—. Treatment of exophthalmic goiter (*E*), 165

WORTH, E. A., (*See* McIntyre, Worth and McIntyre), 706

Z

ZINGHER, ABRAHAM. Practical applications and uses of the Schick test, 117

SUBJECT INDEX

A

Abel's vividiffusion method, modification of, 520
Aborted fungi, 358
Absorption of inhaled substances, route of, 341
Acetone and creatinine in urine, routine determination of, 338
Acute inflammations of the nose, pharynx and tonsils, etiology of, 175, 253, 322
Adrenal glands, James theory of the emotions in relation to, 193
Air motion, in ventilating, 609
Albumins of lung extract, 380
Alkali reserve of blood plasma during acute anaphylactic shock, 555
of blood plasma during protein shock, 481
of blood plasma, technic for titrating the, 38
Amboceptor, natural, removal of, from human sera, 218
Amebic abscess of liver, 211
colitis, in Siam, 210
American relief work in Vienna, 51
Amino acids, effect of, on growth of tubercle bacilli, 96
Anaphylactic colds, 327
shock, alkali reserve of blood plasma during acute, 555
Anemia and resistance of red blood cells, 498
Aneurisms of heart, multiple, 209
Antigen, technic for preparing, 633
Antimony, sodium thioglycollate, 369
Antrum, motility of, and relation of rhythmic activity of the pyloric sphincter to that of the antrum, 124
Apparatus for staining and drying slides, 101
used in the estimation of basal metabolism, 657
Arsenic, sodium thioglycollate, 369
Arsphenamine, silver, 527
treatment of syphilis, complications of, 345
Arterial disease, clinical determination of early, 597
Aspirgillus fumigatus, 357

B

Bacteria, a simple method for isolating from pathologic material, 104
Bacterial infection, spread of, 471
vaccines—chloretone solution as a vehicle for their administration, 47
Bacteriologic studies of acute inflammation of the nose, pharynx and tonsils, 268
Bacteriology of chronic nontuberculous lung disease, 349
of the common cold, 176
Basal metabolism, apparatus used in the estimation of, 657
calculation of, 678
Benign tumors of the gastrointestinal tract, 339
Benzyl benzoate, pharmacological study of, 62
esters, pharmacology of some, 388
Beryllium (glucinum), sodium thioglycollate, 366
Biochemical changes in traumatic shock, 405
Bismuth, sodium thioglycollate, 364
Blood cells, red, resistance of, 454, 487, 568
changes in the case of hemophilia after transfusion, 102
chemical changes in, in disease, 17
chlorides, estimation of, 20
coagulating and smooth muscle-contracting properties of tissue extracts, relation between, 374
coagulation, a preliminary report on, 195
determination of plasma percentage in, 621
distribution of uric acid in the, 680
drawn, stability, of 45
hemoglobin content of, 648
human, precipitin reaction used as evidence for the identification of, in an American court, 709
plasma during protein shock, alkali reserve of, 481
smears, two stains used in preference to Wright's in routine staining of, 593
sugar tolerance in cancer and in hypertension, 227
typed, using Moss's grouping, 276
volume, method for the determination of, 618
whole, estimation of chlorides in, 44
Bloody spinal fluid, value of Ross-Jones test on, 642
Brain stem structures in influenza, 549
Bronchial asthma, 207
B. tuberculosis, methods for examining sputa for, 41

C

Cadmium, sodium thioglycollate, 367
Calculation of basal metabolism, 678
Cancer and hypertension, blood sugar tolerance in, 227

Carcinoma and peptic ulcers of the thyroid in a dog, 213
Cardiorenal disturbances and resistance of red blood cells, 500
Central nervous system in influenza, 548
Cerium, sodium thioglycollate, 368
Chaulmoogra oil in the treatment of tuberculosis, 415
Chemical changes in the blood in disease, 17
Chilling, excessive, as an excitant of infection, 181
interpretation of reactions to, 324
Chloretone solution as vehicle for administration of bacterial vaccines, 47
Chloride content of whole blood, 18
Chlorides, estimation of, in whole blood, 44
Chromium, 370
Chronic nontuberculous lung disease, bacteriology of, 349
Ciliary ganglion, exposure of, in dog, for experimental work, 639
utilization of, for class work in physiology and pharmacology of the eye, 161
Classification of streptococcus, 312
Clinical determination of early arterial disease, 597
synthesis, and syphilology, 469
Coagulable protein in serum, determination of, 223
Coagulant solution, purified active, 383
Coagulation, blood, preliminary report on, 195
of blood, substances which accelerate, 198
of blood, substances which retard, 198
Cobalt, sodium thioglycollate, 371
Cold, common, and weather and ventilation, 603, 684
bacteriology of, 176
Colorimetric method for the estimation of morphine in colloidal mixtures and tissues, 699
of determination of lactose in milk, 221
Comparative values of complement-fixation methods in syphilis, 233
Complement-fixation methods in syphilis, comparative values of, 233
observations of the quantitative nature of, 144
vs. amboceptor titrations in the Wassermann test, 153
Complications of arsphenamine treatment of syphilis, 344
Composite reagent for the determination of sodium chloride in urine, 160
Control of measles, 114
Copper, sodium thioglycollate, 365
Creatinine and acetone in urine, routine determination of, 338
Crude antithrombin solution, 379

D

Determination of blood volume, method for, 618
Diagnosis of typhoid and paratyphoid infections, 335
Dietaries, deficient, effects of, on monkeys, 170
Dietetic treatment of diabetes mellitus, 57
Diphtheria, active immunization of infants against, 120
in the army, 28
Diseases in animals communicable to man, 594
Distribution of uric acid in the blood, 680
Duration of systole and diastole under different cardiovascular conditions, 298

E

Encephalitis lethargica, late sequelae of, 288
Epidemic control, principles of, 35
of influenza, report of, in army post of American Expeditionary Forces in France, 78
respiratory diseases in the army, personal experiences with, 25
Epidemiology of tuberculosis, 231
Estimation of basal metabolism, apparatus used in, 657
Etiology of acute inflammations of the nose, pharynx and tonsils, 175, 253, 322
of scarlet fever, 561
Exophthalmic goiter, treatment of, 165
Experimental influenza bacillus infection in man, 525
Extracts of fresh normal tissue, 378

F

Fibrinogen splits in the process of clotting, evidence that, 196
Freshness and odor in rooms, 607
Functional anatomy of pars pylorica, 126

G

Gas analysis, principles of, 669
Gasometer for use in estimation of basal metabolism, 664
Gas-sampling bottles for use in estimation of basal metabolism, 667
Gastric carcinoma, relation of differentiation and lymphocytic infiltration to postoperative longevity in, 473
Gastrointestinal tract, benign tumors of the, 339
Glands of internal secretion in influenza, 548
Goiter, exophthalmic, treatment of, 165
in Northern Michigan, 719
Gold, sodium thioglycollate, 366

H

Heart and great vessels in influenza, 537
block, a simple device for the demonstration of, in the student laboratory, 635
Hecht-Gradwohl test, 236
employing ice chest fixation, 706

Hemoglobin content of the blood, 648
solution, preparation of, for injection, 619
Hemolytic streptococci, 316
Hemophilia, after transfusion, blood changes in, 102
Hemostatic agents, 398
and the spontaneous changes in coagulation time following hemorrhage, 59
Hormone, specific, of posterior portion of pituitary gland, isolation of, 48
Humidity, 606
Hyperthyroidism, roentgen ray therapy in, 284

I

Ice water-bath in complement fixation for the Wassermann reaction, a shortened technic, 392
Immunization of infants against diphtheria, 120
Immunized blood, transfusion with, in a case of infectious endocarditis, 191
Immunology, present day, 229
Infection, excessive chilling as an excitant of, 181
Inflammations, acute, of the nose, pharynx, and tonsils, etiology of, 175, 253, 322
due to systemic toxic, and neurotic factors, 327
Influenza among the Lapps, 57
and pneumonia in Siam, 206
and tuberculosis, 105, 651
bacillus infection in man, experimental, 525
epidemic, report of, in an army post in the American Expeditionary forces in France, 78
in the army, 30
pathology of, in those with chronic mental disease, 531
Institutional practice of medicine, relation of pathologist to, 331
International organization and public health, 529
Internist and roentgenology, 53
Interpretation of the Wassermann test, 579
Intestinal parasites in Siam, 209
Isolating bacteria from pathologic material, a simple method, 104
Isolation of the specific hormone of posterior portion of pituitary gland, 48

J

James theory of the emotions in relation to adrenal glands, 193
Jaundice and resistance of the red blood cells, 496
in atrophic cirrhosis of liver, 209

K

Kidneys in influenza, 540

L

Laboratory examinations and records, a system of, 505
records, 515
technician, training and proper recognition of, 644
Lactose, determination of, in milk by colorimetric method, 220
Late sequelae of encephalitis lethargica, 288
Lead, excretion of, 448
pharmacologic action of, in organic combination of, 427
poisoning, 496
clinical symptoms of, 427
sodium thioglycollate, 368
Lipoidal antigens for the Wassermann test, standard method for preparing and standardizing, 624
Liver in influenza, 539
Lobar pneumonia, 209
Lungs in influenza, 533
Lymphosarcoma, primary, of the stomach, 249

M

Malaria and the resistance of the red blood cells, 490
Malignant growths and resistance of the red blood cells, 501
Manganese, sodium thioglycollate, 371
Mask for estimation of basal metabolism, 662
Measles, control of, 114
in the army, 26
Mercury manometer float that will ride on the surface of the mercury at all times, 710
sodium thioglycollate, 367
Metal salts of thioglycollic acid, preparation of, 359
Mexican smallpox, 289
Modification of Van Slyke's titration method for estimating the alkali reserve of blood, 37
Molybdenum, sodium thioglycollate, 370
Morphine, a colorimetric method for estimation of, in colloidal mixtures and tissues, 699
Moss's grouping, report on five thousand blood types, using, 276
Motility of the antrum and relation of rhythmic activity of pyloric sphincter to that of the antrum, 125
Mucors, pathogenic, 356
Muscle-contracting and blood-coagulating properties of tissue extracts, relation between, 374
Myxedema, life-history of first case treated with thyroid extract, 55

N

Negative Wassermann reaction in syphilis, 653
Nickel, sodium thioglycollate, 371
Nonhemolytic streptococci, 316

P

Paratyphoid and typhoid infections, diagnosis of, 335
Pars pylorica, functional anatomy of, 126
Pathologists, relation of, to institutional practice of medicine, 331
Pathology of influenza as seen in those with chronic mental disease, 531
of lymphosarcoma of the stomach, 250
Pellagra, two recent papers on, 654
Penicillium glaucum with modified sporangium, 356
Peptic ulcer and carcinoma of the thyroid in a dog, 213
Permeability of corpuscles to added uric acid, 682
Pharmacologic action of lead in organic combination, 427
of salvarsan, 1
study of benzyl benzoate, 62
Pharmacology of benzyl esters, 388
Plasma percentage in blood, determination of, 621
Platinum, sodium thioglycollate, 372
Polyvalent antipneumococcic serum, 23
Postmortem examinations in Siam, analysis of, 199
Pneumonias, 614
review of ninety-four necropsies, 611
Precipitin reactions used as evidence for the identification of human blood in an American court, 709
Pregnancy and the resistance of the red blood cells, 487
Preparation and standardization of polyvalent antipneumococcic serum, 23
Primary lymphosarcoma of the stomach, 249
Provocative Wassermann, 590
Public health and international organization, 529

Q

Quantitative nature of complement fixation, 144

R

Recording laboratory reports on history, 511
Red blood cells, resistance of, 454, 487, 568
Reinfection, tuberculosis and, 162
Relation of differentiation and lymphocytic infiltration to postoperative longevity in gastric carcinoma, 473
Renal insufficiency, test for early, 463
Research information bureau, 230
Resistance of the red blood cells, 454, 487, 568
and malaria, 490
during pregnancy, 487
in anemia, 498
in cardiorenal disturbances, 500
in jaundice, 496
in malignant growths, 501
in syphilis, 494
in tuberculosis, 492
in typhoid fever, 493
Respiratory laboratory, 660
Rhinitis, acute, 328
Roentgen ray therapy in hyperthyroidism, 284
Roentgenology and the internist, 53
Ross-Jones test on bloody spinal fluid, value of, 642
Rubidium, sodium thioglycollate, 365

S

Salvarsan, pharmacological action of, 1
Scarlet fever, etiology of, 561
in the army, 28
Schick test, factors influencing reliability of, 118
practical applications and uses of, 117
technic of, 119
Scurvy in Northern Russia, 168
Sensation and moisture, 609
Serology of scarlet fever, 566
Shaking devise, simple, 222
Shock, traumatic, biochemical changes in, 405
Sickness rates in individual rooms and schools, 684
Silver arsphenamine, 527
sodium thioglycollate, 365
Sinusitis, purulent, 614
Smallpox, are there two diseases under present diagnosis of, 54
Sodium bicarbonate, effect of administration of, on shock, 484
chloride, composite reagent for the determination of, in urine, 160
Spleen in influenza, 540
Sputa, methods for examining, for B. tuberculosis, 41
Stability of drawn blood, 45
Staining and drying slides, apparatus for, 101
Stains used in preference to Wright's stain in routine staining of blood smears, 593
Streptococci isolated from normal and pathologic throats, 315
Streptococcus, classification of, 312
Superinfection in syphilis in its relation to subtreatment, 717
Syphilis and resistance of the red blood cells, 494
superinfection in, in its relation to subtreatment, 717
Syphilology and clinical synthesis, 469
System of laboratory examinations and records, 505
Systole, method for determining duration of, 292
ventricular, factors modifying duration of, 291

T

Tellurium, 370
Test for early renal insufficiency, 463
Thallium, sodium thioglycollate, 367
Thioglycollic acid, preparation of metal salts of, 359

Titanium, 368
Toxin-antitoxin, mixtures of, used for immunization, 121
Training and proper recognition of the laboratory technician, 644
Transfusion with immunized blood in case of infectious endocarditis, unsuccessful result following, 191
Tubercle bacilli, effect of amino acids on growth of, 96
Tuberculosis and influenza, 105, 651
and reinfection, 162
and resistance of the red blood cells, 492
chaulmoogra oil in the treatment of, 415
in Siam, 206
what we know and what we do not know about, 112
Tumors, benign, of the gastrointestinal tract, 339
in Siam, 207
Tungsten, sodium thioglycollate, 370
Typhoid and paratyphoid infections, diagnosis of, 335
fever and resistance of the red blood cells, 493
in Siam, 208
Typhus fever, are we in danger of, 347

U

Uranium, sodium thioglycollate, 370
Uric acid, distribution in the blood, 680

V

Vaccines, effects of, on nontuberculous disease of the lung, 353
Vanadium, sodium thioglycollate, 369
Van Slyke's titration method for estimating the alkali reserve of blood, 37
Variations in the Wassermann reaction, 98
Vasomotor reactions of human subjects to chilling of body surface, experimental studies of, 184
to chilling, 253
Ventilation, present day method of studying, 521
weather, and the common cold, 602, 684
Ventricular systole, factors modifying duration of, 291
Vitamine, notes on, 711
Vividiffusion method of Abel, modification of the technic of, 520

W

Wassermann reaction, ice water-bath in complement fixation for the, 392
negative in syphilis, 653
variations in, 98
test, and its interpretation, 579
complement vs. amboceptor titrations in, 153
in pregnancy, 590
standard method for preparing and standardizing lipoidal antigens for, 624
Weather, ventilation, and the common cold, 602, 684

Z

Zinc, sodium thioglycollate, 372

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